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SYNTHESIS, STRUCTURE-ACTIVITY RELATIONSHIPS
AND MOLECULAR MODELLING
OF HISTAMINE H₃ RECEPTOR LIGANDS

IWAN J. P. DE ESCH

1620152

**Synthesis, structure-activity relationships
and molecular modelling
of histamine H₃ receptor ligands**

The research described in this thesis was funded by a grant of the Netherlands Technology Foundation (STW) and performed at the division of Medicinal Chemistry, Department of Pharmacochimistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands. The Department of Pharmacochimistry is an integral part of the Leiden/Amsterdam Center for Drug Research (LACDR). The modelling studies that are described in chapter 8 were performed at the Drug Design Group, University of Cambridge, UK.

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Synthesis, structure-activity relationships and molecular modelling of histamine H₃ receptor ligands

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Iwan Jozef Philomena de Esch

geboren te Heerlen

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mr.dr. P.H.J. Nederkoorn



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Chapter 1

Introduction

Parts of this chapter have been published:
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In *The histamine H₃ receptor; a target for new drugs*. Rob Leurs and Henk Timmerman Eds.;
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1.1 Histamine and its receptors.

Histamine is not only found in many plants but is also present in nearly all animal tissues where it is detected in many cell lines, *e.g.*, mastcells, basophils, platelets, endothelial cells and neurons.¹ The small compound has a role as chemical messenger, *i.e.*, it transfers signals from one cell to another. As such, it is able to induce numerous (patho)physiological effects, like smooth muscle contraction, stimulation of hormone release, modulation of immune responses, gastric acid secretion, induction of sleep and modulation of cognitive processes (for a thorough review, the reader is referred to the literature¹). Histamine is notoriously famous for its role in allergic conditions and inflammation as it leads to the well known symptoms of irritation in skin and airways.^{2,3} In 1937, Bovet and Staub discovered compounds that antagonised the effect of histamine on these allergic reactions.⁴ Subsequently, many other chemicals that have similar actions have been identified. Some of these compounds (*e.g.*, mepyramine, diphenhydramine) were introduced for clinical use in allergic conditions like hay fever. In the sixties, Ash and Schild observed that some antagonists cannot block the effects of histamine on gastric acid secretion nor the vascular effects.⁵ Therefore, they suggested that histamine acts via two distinct receptor subtypes. This theory was validated when Black and co-workers developed the compound burimamide.⁶ This ligand could inhibit the effect of histamine on the gastric acid secretion and the vascular systems, but did not inhibit the aforementioned allergic reactions. This led to a classification of histamine receptors. It was postulated that histamine's effect in allergic reactions (amongst others) was mediated by H₁ receptors and its effect on gastric acid secretion (amongst others) by H₂ receptors. Although histamine is the endogenous ligand for both receptor subtypes, the structural requirements for compounds for having affinity on the H₁ or H₂ receptors differ. Thus, selective H₂ ligands could be developed and H₂ antagonists became blockbuster drugs for the treatment of gastric ulcers (*e.g.*, cimetidine, ranitidine).

Besides the (patho)physiological roles of histamine mentioned in the previous subsection, it was long speculated that the biogenic amine has a role as a neurotransmitter. Histamine is widely distributed within the mammalian central nervous system (CNS). Mapping of the histaminergic pathways in rat and guinea-pig revealed that the arrangement of the histaminergic fibres were similar to *e.g.*, the noradrenaline and 5-hydroxytryptamine pathways.^{7,8} The role of histamine as a neurotransmitter became apparent in 1983 when Arrang and co-workers discovered that histamine inhibits its own release from depolarized slices of rat cortex.⁹ Such feedback mechanisms mediated through presynaptic receptors are crucial to neurotransmission. These effects were found not to be mediated by H₁ or H₂

receptors since neither the H₁ nor the H₂ activity of the histaminergic ligands correlated with the inhibitory effect. Hence, the results indicated a third histamine receptor subtype. In 1987, the existence of this H₃ receptor was validated by the development of the potent and selective H₃ antagonist thioperamide.¹⁰ Subsequent pharmacological characterization of the receptor using this and other selective H₃ ligands that have been discovered, indeed established the H₃ receptor as being located on the presynaptic neuron endings (see subsection 1.4 for more details).

The histamine H₃ receptor has been referred to as the last receptor revealed by classical pharmacology.¹¹ Since the late eighties, new receptors are discovered, almost on a weekly basis, by molecular biology techniques. The rapid cloning of new receptor genes has even led to orphan receptors for which no pharmacological and (patho)physiological function, nor endogenous ligand is known. On the contrary, the H₃ receptor, as mentioned, was revealed by measuring a unique pharmacological effect under the influence of large series of compounds. In fact, H₃ ligand-based information has, for a long time, been more abundant than any other H₃ related information. In section 1.2, a review will be given of the structural requirements for H₃ ligands. Subsequently, in section 1.3, the limited molecular modelling studies that have been described for H₃ ligands will be summarised. Finally, in section 1.4, the relevant pharmacological and (patho)physiological aspects of the histamine H₃ receptor will be discussed

1.2 Structural requirements for histamine H₃ receptor ligands.

In the following subsections, an up-to-date overview of the ligands that have activity on the H₃ receptor is presented. In order to be as complete as possible, we also describe compounds that emerged from investigations described in this thesis; these ligands are discussed in more details in following chapters. It is the intention of this overview to focus on the *in vitro* activity and to illustrate the structure activity relationships (SARs) of the H₃ ligands that form the foundation for the work described in this thesis. However, a thorough identification of the SARs is ambiguous for various reasons. Comparison of compounds within one series, being tested on the same assay, can be ambiguous as the reported standard errors are sometimes quite significant. Even more troublesome is comparison of the biological data obtained using different pharmacological assays, as stunning differences in activity of ligands tested using different assays have been reported (*vide infra*). Therefore, the activities and affinities are always presented with a reference to the test system that was used. Last but not least, comparison within and between different classes of compounds is sometimes hampered

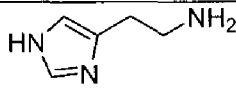
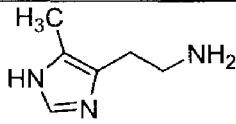
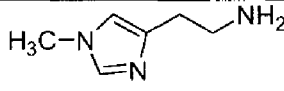
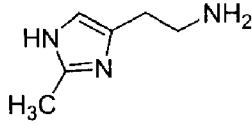
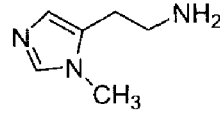
because the series are often not complete or, for various reasons, comparable. Nevertheless, certain trends can be identified.

1.2.1 Histamine H₃ receptor agonists.

Histamine derivatives

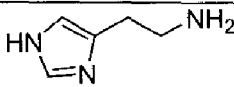
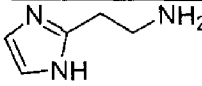
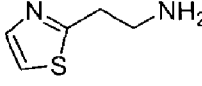
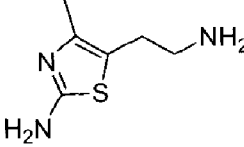
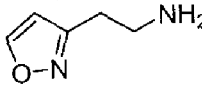
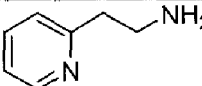
Histamine (**1**) mediates its actions via three receptor subtypes, the postsynaptic (or hormonal) H₁ and H₂ receptors and the presynaptic H₃ receptor. The endogenous ligand has higher affinity for the latter, thus being more selective for the receptor subtype that has a regulatory role in the synthesis and release of the neurotransmitter. In the search for selective H₃ agonists, the endogenous compound histamine has been used as a lead structure. It has been shown that the 4-substituted imidazole moiety is essential for agonistic activity. Whereas additional substitution on the imidazole ring of histamine can lead to interesting H₂ (*e.g.*, 4(5)-methylhistamine (**2**)) and H₁ (*e.g.*, 2-methylhistamine (**4**)) agonists, such modifications result in complete loss of H₃ activity (table 1).^{9,12} Furthermore, replacement of the imidazole moiety by other heterocycles or other potential bioisosteric (for H₁ or H₂) replacements is not allowed for H₃ agonism, as is illustrated in table 2.^{9,12,13} This indispensable role of the imidazole ring implies that the interaction of this moiety with the receptor site is highly compulsory, and two hydrogen bonds between the imidazole moiety and the receptor in addition to an aromatic interaction can be anticipated.

Table 1. H₃ activity of histamine and imidazole ring methylated analogues.

No.	Compound	Structure	pD ₂ ^a
1	histamine		7.4
2	4(5)-methylhistamine		< 3.0
3	N ^ε -methylhistamine		< 6.0
4	2-methylhistamine		< 4.3
5	N ^π -methylhistamine		< 6.0

^a K⁺-Stimulated [³H]-histamine release from rat cortex.

Table 2. Histamine H₃ receptor activity of imidazole substituted histamine analogues.

No.	Compound	Structure	pD ₂ ^a
6	histamine		7.4
7	2-(2-aminoethyl)imidazole [H ₂ agonist]		< 4.0
8	2-(2-aminoethyl)-thiazole [H ₂ agonist]		< 3.0
9	amthamine [H ₂ agonist]		4.7 ^b
10	3-(2-aminoethyl)-isoxazole [H ₁ agonist]		< 4.0
11	2-(2-aminoethyl)-pyridine [H ₁ agonist]		< 4.0

^a K⁺-Stimulated [³H]-histamine release from rat cortex.^b Electrically evoked contractions of guinea-pig ileum.

Whereas alteration of the imidazole unit is not allowed, modification of the side chain can result in very potent and selective H₃ agonists. Methylation of the α -position leads to enantiomeric compounds that reveal a stereoselectivity of the H₃ receptor (table 3).⁹ Whereas the eutomer (most active enantiomer) (R)- α -methylhistamine (**12**) has an activity that is about ten times higher than the endogenous ligand (**1**), the distomer (S)- α -methylhistamine (**13**) is about 15 times less active than histamine. (R)- α -methylhistamine (**12**) is often used as a standard agonist for pharmacological studies involving H₃ receptors, as it is not only very potent but also very selective (about 20,000 times more potent on H₃ than on the H₁ and H₂ receptors).

Methylation at both the α and β -position of the ethylene side chain is allowed.¹⁴ (R) α ,(S) β -Dimethylhistamine (**14**) is a potent and very selective agonist as it is about 100,000 times more active at the H₃ receptor than at the H₁ and H₂ receptor. Methylation of the β -position is synthetically difficult and the resolution of β -methylhistamine (**17**) has not yet been described. Pharmacological data using the racemic mixture **17** indicates that a methyl group can be accommodated in this position.¹⁵ The histamine derivative with two methyl groups in the α -position (**18**) is also a potent agonist. However, double methylation at the β -position results in the very weak agonist **19**.¹⁵ Increasing the Van Der Waals radius of the substituents on either the α - or β -position of the ethylene side chain is not allowed (**20-22**).¹²

Table 3. H₃ activity of ethylene side chain substituted histamine analogues.

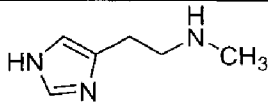
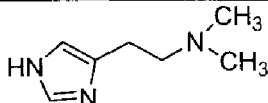
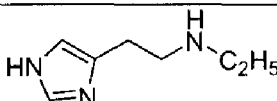
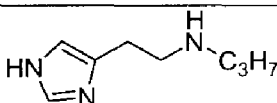
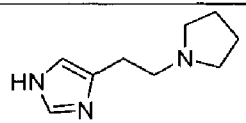
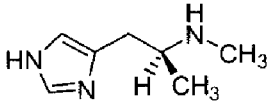
No.	Compound	Structure	pD ₂ ^a
1	histamine	-	7.4
12	(R)- α -methylhistamine		8.4
13	(S)- α -methylhistamine		6.3
14	(R)- α , (S)- β -dimethylhistamine		8.5
15	(S)- α , (R)- β -dimethylhistamine		6.5
16	(\pm)-threo- α , β -dimethylhistamine		6.7
17	(\pm)- β -methylhistamine		7.7
18	α , α -dimethylhistamine		7.6
19	β , β -dimethylhistamine		5.8
20	(R)- α -chloro-methylhistamine		4.7
21	(S)- α -chloro-methylhistamine		5.9
22	(\pm)- β -ethylhistamine		5.0
23			

^a K⁺-Stimulated [³H]-histamine release from rat cortex.

Krause and co-workers have developed very potent and effective azomethine prodrugs of (R)- α -methylhistamine (**12**).¹⁶ One of these prodrugs, **23**, is very efficient for delivering a high CNS level of (R)- α -methylhistamine (**12**), and is therefore useful as a pharmacological tool and may become an H₃ histaminergic drug for therapeutic use.

The amino group of histamine has been alkylated as well (table 4). It was found that N ^{α} -methylhistamine (**24**) is about 2.5 times more active than histamine (**1**).¹⁰ Double methylation of the amino group, leading to **25** is also allowed.¹⁷ As with substituents on the ethylene spacer, larger substituents on the amino group of histamine results in diminished H₃ activity. (**26-28**),¹⁸ indicating that the available space in the agonistic binding site is very limited. Remarkably, it has been reported that (R) α ,N ^{α} -dimethylhistamine (**29**) has a low H₃ activity, although both (R) α -methylhistamine (**12**) and N ^{α} -methylhistamine (**24**) are very potent agonists.¹⁹ These findings have not yet been rationalised.

Table 4. Histamine H₃ activity of N ^{α} -alkyl substituted histamine analogues.

No.	Compound	Structure	pD ₂ ^a
24	N ^{α} -methylhistamine		7.8 ^b
25	N ^{α} ,N ^{α} -dimethylhistamine		7.6
26	N ^{α} -ethylhistamine		7.1
27	N ^{α} -propylhistamine		< 5.2
28	N-[2-(1H-imidazol-4-yl)ethyl]-pyrrolidine		6.2 (α =0.6)
29	(R) α ,N ^{α} -dimethylhistamine		5.8

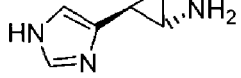
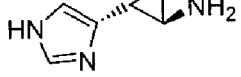
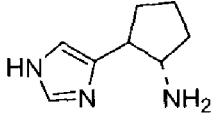
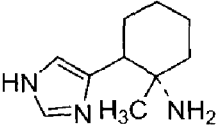
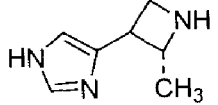
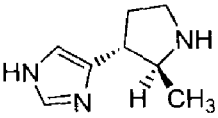
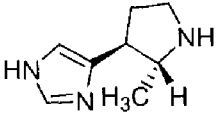
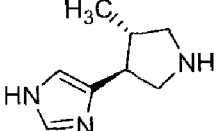
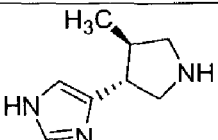
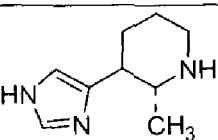
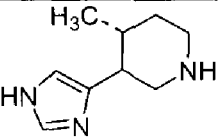
^a K⁺-Stimulated [³H]-histamine release from rat cortex.

^b Electrically evoked contractions of guinea-pig ileum.

Both (R)- α -methylhistamine (**12**) and N $^{\alpha}$ -methylhistamine (**24**) have been tritiated and used to characterise the histamine H₃ receptor in binding assays.¹⁰ However, the involvement of G-protein coupling in agonist binding, and hence, the putative presence of two affinity states hampers straightforward use of agonists in these studies.²⁰ To circumvent these problems, labelled antagonists have been developed subsequently (*vide infra*).

Several histamine analogues have been reported in which the flexible side chain is incorporated in a ring structure (table 5). In chapter 3, the synthesis and activity of the distinct *trans*-cyclopropylhistamine enantiomers is described.²¹ (1S,2S)-Cyclopropylhistamine (**30**) is considerably more active than its enantiomer (1R,2R)-cyclopropylhistamine (**31**). Incorporation of the α and β carbon atoms of the ethylene linker of histamine in larger ring systems leads to the low activity compounds **32-33** (both tested as racemic mixtures of their respective *trans*-isomers).²² Incorporation of the basic amino group in side chain ring systems can lead to potent H₃ agonists. It has been reported in patent literature that the racemic mixture of *trans*-substituted azetidine **34** has a high H₃ affinity.²³ Increasing the ring size from a four to five membered ring leads to the stereospecific pyrrolidine analogues **35-37**.^{22,23} These compounds were prepared as rigid analogues of (R)- α -methylhistamine (**12**) and give valuable information about the biologically active conformation of H₃ ligands (*vide infra*). SCH49648 (**36**) and SCH50971 (**38**) are very potent H₃ agonists with an excellent *in vivo* receptor selectivity.²⁴ Interestingly, these substituted pyrrolidine-containing ligands can be considered as (R)- α -methyl analogues with ethyl substituents on the β -position (or, alternatively, on the N $^{\alpha}$ -position). Although these substituents are not allowed on the flexible side chain analogues (see **22** and **26**, respectively), incorporation of larger substituents in a ring system does not hinder receptor binding. Further enlargement of the ring results in the low affinity compounds **39** and **40**,²³ indicating that these ligands cannot adopt the desired conformation or, alternatively, that these bulkier structures can not be accommodated in the binding site.

Table 5. Histamine H₃ activity and affinity of conformationally restrained histamine analogues

No.	Compound	Structure	pD ₂ ^a	pK _i ^b
30	(S) α , (S) β -cyclopropylhistamine		7.1 (α =0.71)	7.6 ^c
31	(R) α , (R) β -cyclopropylhistamine		5.8 (α =0.64)	8.7 ^c
32				6.1
33				< 5.7
34				8.2
35	SCH 49647 (2S,3R)			7.5
36	SCH 49648 (2R,3S)		7.1	8.5
37	SCH 50972 (3S,4S)			7.5
38	SCH 50971 (3R,4R)		7.5	8.6
39	(±)			< 5.7
40	(±)			6.4

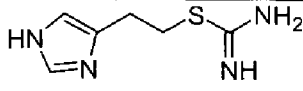
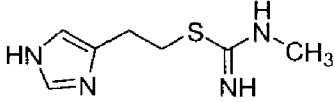
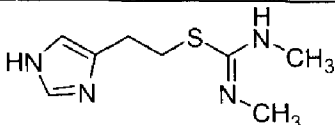
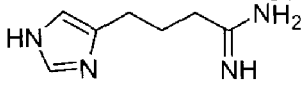
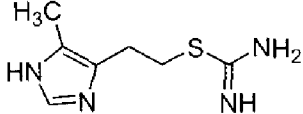
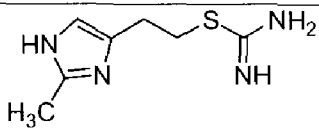
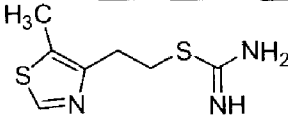
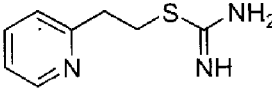
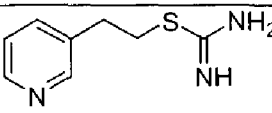
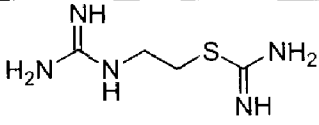
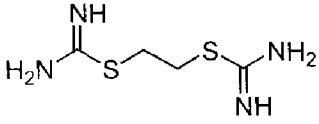
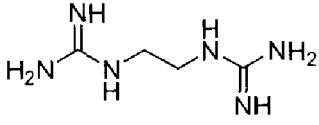
^a Electrically evoked contractions of guinea-pig ileum.^b Displacement of [³H] N^α-methylhistamine from guinea-pig brain tissue.^c Displacement of [³H] N^α-methylhistamine from rat cortex.

Imetit derivatives

Substitution of the amino group of histamine by an isothioureia moiety resulted in the highly potent and selective agonist imetit (**41**) (table 6).^{25,26,27,28} In contrast to methylation of the amino group of histamine (**1**), which is allowed for activity (see **24**), methylation of isothioureia moiety drastically reduces activity (**42**).^{26,28} Double methylation leads to the antagonist **43**. The sulfur atom of imetit does not seem to be important for receptor binding since the amidine analogue SKF91606 (**44**) is even more potent than imetit (**41**).²⁶

Compared to histamine (**1**), the linker connecting the imidazole ring and the proton-donating nitrogen atom of imetit (**41**) and SKF92606 (**44**) is longer. As will be described in a following subsection, elongation of the ethylene linker of histamine results in compounds with antagonistic H₃ activity (table 11, *vide infra*). In this light the agonistic activity of imetit may be a surprise. In chapter 4 of this thesis is the description how both nitrogen atoms of the isothioureia and amidine moiety of imetit (**41**) and SKF 91606 (**44**), respectively, form strong hydrogen bonds with the carboxylate of an interacting aspartic-acid residue of the receptor. This interaction, different from the interaction of an amino group with a carboxylate, induces a folded conformation of imetit (**41**) and SKF 91606 (**44**), thereby enabling a strong interaction with the agonistic binding site. As with histamine (**1**), replacement of the imidazole ring of these highly potent imetit derivatives results in analogues that lack H₃ activity (**45-52**).^{25,29}

Table 6. Histamine H₃ receptor activity of imetit and analogues.

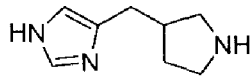
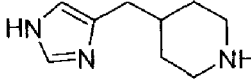
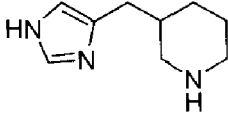
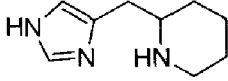
No.	Compound	Structure	pD ₂ ^a
41	imetit		8.1 9.0 ^b
42	VUF 8621		7.8 ^b
43	VUF 8973		pA ₂ =7.3 ^b
44	SKF 91606		9.0
45	VUF 9032		6.0 (α =0.9)
46	VUF 8974		pA ₂ =5.4
47	VUF 8844		inactive ^c
48	VUF 8863		6.0 ^c (α =0.5)
49	VUF 8848		inactive ^c
50	VUF 8430		5.9 ^c (α =0.5)
51	VUF 8332		pA ₂ =6.0 ^c
52	VUF 8309		inactive ^c

^a Electrically evoked contractions of guinea-pig ileum.^b K⁺-Stimulated [³H]-histamine release from rat cortex.^c Electrically-stimulated [³H]-histamine release from rat cortex.

Immepip derivatives

As will be described in a following subsection, elongation of the flexible spacer connecting the imidazole ring and the basic moiety in the side chain of potent agonists like histamine (**1**) and imetit (**41**) results in loss of agonistic activity (table 11). However, if an elongated side chain is incorporated into a ring structure, potent H₃ agonists may be obtained (table 7). The racemic mixture of (±)-3-(1*H*-imidazol-4-yl-methyl)pyrrolidine (VUF4864, **53**) has considerable H₃ agonistic activity.³⁰ Replacement of the pyrrolidine moiety by a piperidine ring results in the potent and selective H₃ agonist immepip (**54**).³¹ In this compound, the imidazole ring and the basic amino group are separated by four methylene units, but, nevertheless, the ring system seems able to adopt the proper conformation for binding to the agonistic binding site. Replacement of the 4-piperidine ring of immepip (**54**) by a 3-piperidine ring or a 2-piperidine ring (leading to **55** and **56**, respectively), results in diminished agonistic activity.³⁰

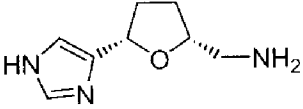
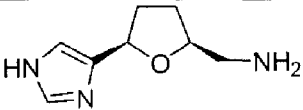
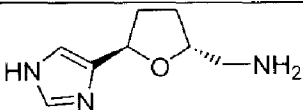
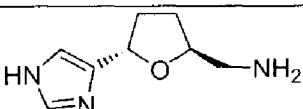
Table 7. Histamine H₃ receptor activity of immepip and analogues.

No.	Compound	Structure	pD ₂ ^a
53	(±)-VUF 4864		7.3 (α=0.8)
54	immepip		8.0
55	VUF 4858		pA ₂ =6.5
56	VUF 4888		pA ₂ < 5.0

^a Electrically evoked contractions of guinea-pig ileum.

Recently, Harusawa and co-workers have described a novel H_3 agonist.³² Four stereoisomers (**57-60**) of a tetrahydrofuran-containing compound were synthesised and their respective H_3 pharmacology was studied using *in vivo* (rat) brain microdialysis (table 8). Imifuramine (**59**) was identified as an H_3 receptor agonist, as it decreases histamine release, an effect that could be blocked by selective H_3 antagonists.

Table 8. Histamine H_3 receptor *in vivo* activity of imifuramine and stereoisomers.

No.	Structure	Compound	reduction of histamine release ^a
57		2S,5R	0%
58		2R,5S	0%
59		2R,5R imifuramine	70%
60		2S,5S	0%

^a Effect after administration of 10 μ M compound.

It has been noted by the authors that imifuramine (**59**), having the *trans*-configuration, is not able to form an intramolecular hydrogen-bond between the cationic amino group and the imidazole moiety, thereby validating the finding presented in chapter 3 and chapter 4 of this thesis that such a hydrogen bond is not important for histamine H_3 receptor agonism.

1.2.2 Histamine H₃ receptor antagonists

Derivatives of histamine as H₃ antagonists

Ganellin and co-workers have shown that attachment of heterocyclic rings to the amino group of histamine results in H₃ antagonists with moderate activity, as shown in table 9.³³ For this particular class of compounds, the amino group is not involved in binding to the receptor protein as this moiety can be replaced by a sulfur atom, resulting in even more potent compounds (compare **65** and **66**). It has been revealed that the nitrogen atom in the pyridine ring of **61-66** is not important for H₃ affinity either, as phenyl-derivatives **67-70** have comparable activities.³⁴ A similar study, but using different heterocycles, has been published by Plazzi *et al.*³⁵ The potent antagonists **71-74** confirm that the hydrogen-donating nitrogen atoms in the side chain are not important for this class of compounds, since omitting these atoms does not affect the H₃ antagonistic activity (compare **71** and **73**).

Table 9. H₃ receptor antagonistic activity of histamine derivatives.

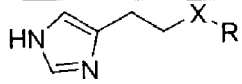
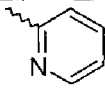
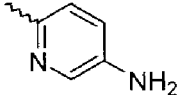
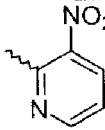
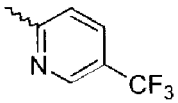
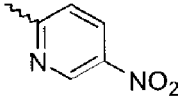
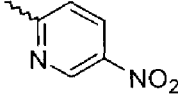
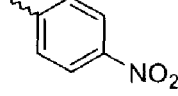
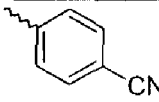
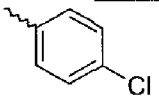
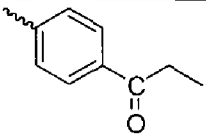
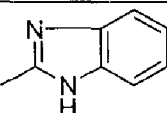
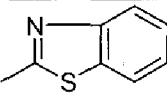
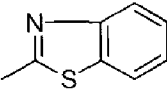
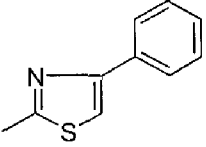
			
No.	X	R	pA ₂ ^a
61	NH		6.7
62	NH		6.7
63	NH		6.6
64	NH		7.8
65	NH		7.5
66	S		8.3
67	S		8.0

Table 9 (continued)

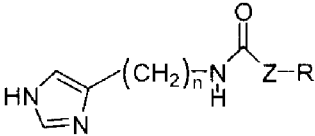
68	O		8.0
69	O		7.7
70	O		8.4
71	NH		7.4 ^b
72	NH		7.7 ^b
73	S		8.0 ^b
74	S		7.8 ^b

^a K⁺-Stimulated [³H]-histamine release from rat cortex.

^b Inhibition of [³H]-N^α-methylhistamine binding to rat cortex.

Clearly, substituents on the amino group of histamine that are bigger than a methyl group hamper binding to the agonistic binding site. Thus, acylation of the amino group of histamine results in H₃ antagonists (table 10).³⁶ In particular, introduction of a lipophilic group, *e.g.*, phenyl or a cycloalkyl groups, separated from the polar amide group by an alkyl spacer results in antagonists, however with moderate activity only.^{37,38} For binding to the antagonistic site, the distance of the lipophilic terminus relative to the imidazole ring seems to determine activity. The position of the amide group is less important (compare **79** and **82**).

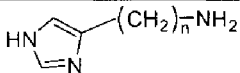
Table 10. Histamine H₃ activity of some N^α-acylated histamine analogues.

				
No.	n	Z	R	pA ₂ ^a
75	2	-	methyl	5.9
76	2	CH ₂	phenyl	6.0
77	2	(CH ₂) ₂	phenyl	6.2
78	2	(CH ₂) ₃	phenyl	7.1
79	2	(CH ₂) ₃	cyclohexyl	7.3
80	2	(CH ₂) ₄	phenyl	6.7
81	3	(CH ₂) ₂	phenyl	7.3
82	3	(CH ₂) ₂	cyclohexyl	7.3
83	3	(CH ₂) ₃	cyclohexyl	7.2

^a K⁺-Stimulated [³H]-histamine release from rat cortex.

Vollinga and co-workers have demonstrated that elongation of the ethylene spacer of histamine results in highly potent H₃ receptor antagonists (table 11).³⁹ Insertion of even one extra methylene unit in the spacer results in the antagonist homohistamine (**85**). A clear optimum in antagonistic activity has been revealed for the pentylene spacer, resulting in the very potent and selective antagonist impentamine (**87**). This compound behaves as a pure antagonist on the guinea-pig jejunum and as a partial agonist on mouse brain cortex.⁴⁰ Further elongation of the pentylene spacer results in diminished H₃ activity (**88-90**).

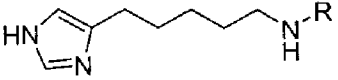
Table 11. H₃ activity of ω-aminoalkyl chain length varied histamine analogues.

			
No.	Compound	n	pA ₂ ^a
84	VUF 8319	1	Inactive
1	histamine	2	pD ₂ =7.4
85	homohistamine	3	5.9
86	VUF 4701	4	7.7
87	impentamine	5	8.4
88	VUF 4732	6	7.8
89	VUF 4733	8	6.0
90	VUF 4734	10	6.0

^a Electrically evoked contractions of guinea-pig ileum.

Attachment of a lipophilic terminus (a so-called lipophilic tail) on the amino group of impentamine (**87**) results in compounds with an increased H₃ activity (table 12).⁴¹ Especially, substitution with a cyclohexyl group or a 4-chlorobenzyl group leads to very potent antagonists, (**92**) and (**96**), respectively. The data suggest that there is a hydrophobic pocket in the H₃ receptor binding site that is available for binding these lipophilic tails of the antagonists.

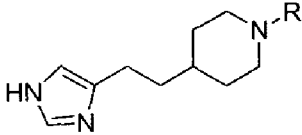
Table 12. Histamine H₃ receptor activity of amino-alkylated impentamine derivatives.

			
No.	Compound	R	pA ₂ ^a
87	impentamine	H	8.4
91	VUF 4904	<i>i</i> -propyl	8.5
92	VUF 4903	cyclohexyl	9.3
93	VUF 5203	Phenyl	8.1
94	VUF 4808	Benzyl	8.8
95	VUF 5299	4-CH ₃ O-benzyl	8.7
96	VUF 5202	4-Cl-benzyl	9.0
97	VUF 5204	Phenylethyl	8.4

^a Electrically evoked contractions of guinea-pig ileum.

In chapter 5 of this thesis, the development of VUF4929 (**98**) is described, a compound that can be considered a ring-closed analogue of impentamine (**87**). Although these two compounds have identical activity on the guinea-pig jejunum, VUF4929 (**98**) reveals a unique activity profile (table 13) not shared by impentamine (**87**) or other H₃ antagonists, since VUF4929 (**98**) is more than ten times less active on the rat cortex. Furthermore, VUF4929 (**98**) reveals a pK_i=6.5±0.1 when displacing the radioligand [¹²⁵I]iodophenpropit and pK_i=7.7±0.1 when displacing the radioligand [³H]N^α-methylhistamine. The remarkable discrepancies between the different pharmacological assays is an indication of differences in effector coupling and of H₃ receptor heterogeneity. Substitution of various lipophilic groups on the piperidine nitrogen atom results in less active compounds (**99-102**). Whereas introduction of cyclohexyl and 4-chlorobenzyl moiety on the amino group of impentamine (**87**) results in up to ten fold more active derivatives (compare **87** and **92**), the analogous modification of VUF4929 leads to a ten fold decrease in activity (compare **98** and **100**), indicating differences in binding mode.

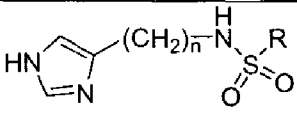
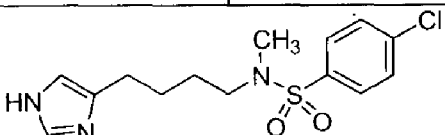
Table 13. Histamine H₃ receptor activity of VUF4929 and derivatives.

			
No.	Compound	R	pA ₂ ^a
98	VUF4929	H	8.4 7.1 ^b pK _i =6.5 ^c pK _i =7.7 ^d
99	VUF5617	CH(CH ₃) ₂	7.4
100	VUF5618	cyclohexyl	7.1
101	VUF4928	benzyl	7.5
102	VUF5619	4-Cl-benzyl	7.8

^a Electrically evoked contractions of guinea-pig ileum.^b Electrically-stimulated [³H]noradrenaline release from rat cortex.^c Displacement of [¹²⁵I]iodophenpropit from rat cortex.^d Displacement of [³H]N^α-methylhistamine from rat cortex.

Recently, novel sulfonamide homologues of histamine have been described by Wolin and co-workers.⁴² The linker connecting the imidazole ring and the nitrogen atom of the sulfonamide moiety, as well as the lipophilic terminus were varied (table 14). A propylene linker results in weak affinity compounds. However, the potency is drastically improved with the introduction of a butylene or pentylene linker. For the butylene spacer, chlorinated phenyl groups used as lipophilic tails seem to be preferred (**108** and **109**). For the pentylene spacer, the 4-*t*-butyl-phenyl and the 4-nitro-phenyl groups are preferred (*e.g.*, **113** and **115**, respectively). The acidic sulfonamide hydrogen atom of the butylene linker derivatives is not involved in hydrogen bonding to the site, since **116**, with a methyl group on the sulfonamide nitrogen atom, is even more potent than **108**.

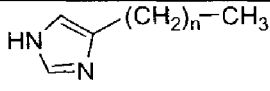
Table 14. Histamine H₃ receptor affinity of some sulfonamides.

			
No.	n	R	pK _i ^a
103	3	4- <i>t</i> -Bu-phenyl	7.3
104	3	4-Cl-phenyl	6.4
105	3	4-NO ₂ -phenyl	7.0
106	4	phenyl	8.0
107	4	4- <i>t</i> -Bu-phenyl	7.8
108	4	4-Cl-phenyl	8.0
109	4	3-Cl-phenyl	8.1
110	4	4-NO ₂ -phenyl	7.8
111	4	phenyl-CH ₂	8.2
112	5	phenyl	7.6
113	5	4- <i>t</i> -Bu-phenyl	8.3
114	5	4-Cl-phenyl	7.9
115	5	4-NO ₂ -phenyl	8.2
116			8.4

^a Displacement of [³H] N^α-methylhistamine from guinea-pig brain tissue.

Experimental evidence showing that for some classes of H₃ antagonists a basic nitrogen atom in the imidazole side chain is not involved in hydrogen-bonding interaction with the receptor, raises questions about the contribution of such a moiety to the affinity of other classes of H₃ ligands. Therefore, the SAR of the lipophilic tail of H₃ antagonists has been explored systematically (see chapter 7).⁴³ The contribution to the H₃ antagonistic activity of simple alkyl groups attached to the imidazole ring was investigated in a first series (table 15). The activity of these moderately active compounds is proportional to the length of the alkyl spacer and no optimum was found. The activity of such simple compounds illustrates the important role of the imidazole ring on H₃ activity.

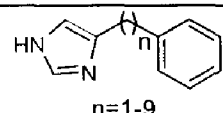
Table 15. H₃ activity of 4-(*n*-alkyl)-1*H*-imidazoles

			
No.	Compound	n	pA ₂ ^a
117	VUF5522	2	6.3
118	VUF5523	3	6.7
119	VUF5524	4	6.4
120	VUF5466	5	6.3
121	VUF5526	6	6.6
122	VUF5467	7	6.8
123	VUF5528	8	7.1
124	VUF5529	9	7.2

^a Electrically evoked contractions of guinea-pig ileum.

In another series, 4-(ω -phenylalkyl)-1*H*-imidazoles were tested for their H₃ activity (table 16). A clear optimum has been found for the pentylene spacer that connects the two aromatic moieties. These results not only indicate the position of a hydrophobic pocket (see chapter 8), but also reveal the contribution of the different structural features of H₃ antagonists on activity. Thus, by comparing impentamine derivative VUF4808 (**94**) (pA₂=8.8) and VUF5517 (**131**) (pA₂=7.1), two compounds with roughly the same distance between the imidazole ring and the phenyl group, it has become evident that the basic nitrogen of VUF4808 (**94**) contributes to the activity and is probably involved in binding to a hydrogen-bonding receptor site point.

Table 16. H₃ activity of 4-(ω -phenylalkyl)-1*H*-imidazoles

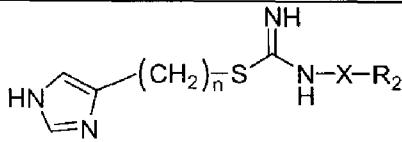
			
No.	Compound	n	pA ₂ ^a
125	VUF5511	1	5.8
126	VUF5512	2	5.9
127	VUF5513	3	6.4
128	VUF5514	4	7.5
129	VUF5515	5	7.8
130	VUF5516	6	7.7
131	VUF5517	7	7.1
132	VUF5518	8	6.5
133	VUF5519	9	6.1

^a Electrically evoked contractions of guinea-pig ileum.

Derivatives of imetit as H₃ antagonists

The potent agonist imetit (**41**) can be converted into ligands with antagonistic activity by attaching a phenyl group to the isothioureia moiety (**134**, table 17).⁴⁴ When separating the phenyl group from the isothioureia group by alkyl chains of various length, an optimum is found for the ethylene spacer, leading to **136**.

Table 17. Histamine H₃ activity of clobenpropit and derivatives.

					
No.	compound	n	X	R	PA ₂ ^a
41	imetit	2	-	H	pD ₂ =8.1 pD ₂ =9.0 ^b
42	VUF 8621	2	-	CH ₃	pD ₂ =7.3 ^b
134	VUF 8397	2	-	C ₆ H ₅	7.0
135	VUF 9028	2	CH ₂	C ₆ H ₅	7.8
136	VUF 9029	2	(CH ₂) ₂	C ₆ H ₅	8.0
137	VUF 9030	2	(CH ₂) ₃	C ₆ H ₅	7.6
138	VUF 9031	2	(CH ₂) ₄	C ₆ H ₅	7.7
139	VUF 8328	3	-	H	8.0
140	VUF 9163	3	CH ₂	c-C ₆ H ₁₁	8.8
141	VUF 9107	3	CH ₂	C ₆ H ₅	8.8
142	VUF 4650	3	CH ₂	C ₆ H ₅ -4-F	9.4
143	clobenpropit	3	CH ₂	C ₆ H ₅ -4-Cl	9.9
144	VUF 5222	3	CH ₂	C ₆ H ₅ -3-Cl	9.3
145	VUF 5223	3	CH ₂	C ₆ H ₅ -2-Cl	9.5
146	VUF 4651	3	CH ₂	C ₆ H ₅ -4-Br	9.8
147	VUF 4652	3	CH ₂	C ₆ H ₅ -4-I	9.2
148	VUF 4658	3	CH ₂	C ₆ H ₅ -4-CH ₃	9.4
149	VUF 4654	3	CH ₂	C ₆ H ₅ -4-OCH ₃	9.4
150	VUF 9151	3	(CH ₂) ₂	C ₆ H ₅	8.8
151	iodophenpropit	3	(CH ₂) ₂	C ₆ H ₅ -4-I	9.6
152	VUF 9152	3	(CH ₂) ₃	C ₆ H ₅	8.3
153	VUF 4571	3	(CH ₂) ₄	C ₆ H ₅	8.5

^a Electrically evoked contractions of guinea-pig ileum.

^b K⁺-Stimulated [³H]-histamine release from rat cortex.

Analogous to the SAR described for histamine derivatives (see table 11), elongation of the ethylene spacer of the agonist imetit (**41**) with an extra methylene unit leads to H₃ antagonism (see VUF8328, **139**).⁴⁴ The antagonistic activity can be further increased by the introduction of lipophilic groups on the isothiourea moiety. The most active compounds in this series have cycloalkyl groups or benzyl groups. Because an aromatic ring can be replaced by a cycloalkyl group (compare **140** and **141**), Van der Goot and co-workers suggested that the interaction of these substructures is through a hydrophobic interaction and not a π - π interaction. Introduction of a 4-chlorobenzyl group on the thiourea moiety results in the extremely potent antagonist clobenpropit (**143**). The enormous activity-increasing effect of a *para*-substituted halogen atom on the benzyl group of the lipophilic tail is not observed in other classes of antagonists (*e.g.*, compare **94** and **96**, table 12) and, therefore, several authors have suggested a different binding mode for the compounds of the clobenpropit class.^{44,45} In chapter 8 of this thesis, a special binding mode for clobenpropit (**143**) is described and it is concluded that the remarkable SAR may be caused by electronic factors.

Using clobenpropit (**143**) as a lead, Menge and co-workers subsequently developed the first selective radiolabelled H₃ antagonist [¹²⁵I]-iodophenpropit (**151**),⁴⁶ a valuable tool for histamine H₃ receptor binding studies.^{47,48}

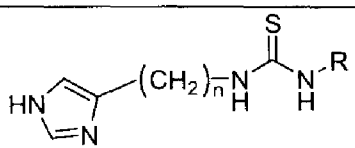
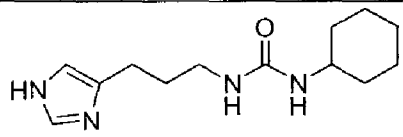
Derivatives of burimamide.

A few years before the identification of the H₃ receptor by Arrang and co-workers,⁹ the antagonistic effect of the H₂ receptor antagonist burimamide (**156**) on the inhibitory action of histamine on electrically evoked contractions of guinea-pig jejunum preparations was described.⁴⁹ Surprisingly, this inhibition was not sensitive to H₂ agonists and, therefore, it was doubtful that the effect was mediated by H₂ receptors. Furthermore, neither H₁ nor adrenergic receptors seemed to be involved. After the discovery of the H₃ receptor, the effect on the guinea-pig jejunum could be assigned to the H₃ antagonistic activity of burimamide. These pharmacological findings have led to the development of a simple and rapid *in vitro* test system for the screening of histamine H₃ ligands.⁵⁰ The compound burimamide did not become a pharmacological tool because of the lack of selectivity. However, Vollinga and co-workers managed to optimize its structure (table 18).⁵¹ Variation of the chain length connecting the imidazole ring and the thiourea moiety has revealed that a pentylene spacer, leading to VUF4613 (**159**), is optimal for H₃ activity and selectivity. Surprisingly, the structure of VUF4613 (**159**) could not be further optimized for H₃ antagonism by attachment of different lipophilic substituents on the thiourea group. The effect of the different, more lipophilic tails on the H₃ activity is minimal, revealing a SAR that is very different from the

SAR revealed for other classes of H₃ antagonists, *e.g.*, the impentamine (table 12) and the clobenpropit (table 17) series. It has therefore been suggested that VUF4613 (**159**) and its derivatives bind to a different, unique binding site of the H₃ receptor.⁵¹

It has also been noted that the replacement of the thiourea group of norburimamide derivative **168** by a urea group (leading to **169**) is allowed for activity.³⁸ These ligands can be considered as flexible analogues of the H₃ antagonist thioperamide (*vide infra*). Remarkably, an analogous replacement of the thiourea group of thioperamide by a urea group is not allowed (see next subsection).

Table 18. H₃ receptor activity of burimamide, VUF4613 and derivatives.

				
No.	compound	n	R	PA ₂ ^a
154	VUF 4577	2	Me	5.5
155	norburimamide	3	Me	6.1
156	burimamide	4	Me	7.1
157	VUF 4613	5	Me	8.0
158	VUF 4740	6	Me	7.9
159	VUF 4613	5	CH ₃	8.0
160	VUF 4614	5	C ₂ H ₅	8.0
161	VUF 4615	5	C ₃ H ₇	7.7
162	VUF 4616	5	(CH ₃) ₂ CH	7.7
163	VUF 4617	5	<i>c</i> -C ₆ H ₁₁	7.5
164	VUF 4618	5	C ₆ H ₅	7.6
165	VUF 4619	5	C ₆ H ₅ CH ₂	7.7
166	VUF 4742	5	(4)-Cl-C ₆ H ₅ CH ₂	8.1
167	VUF 4620	5	C ₆ H ₅ (CH ₂) ₂	7.5
168	VUF 4634	3	<i>c</i> -C ₆ H ₁₁	6.9
169				7.3

^a Electrically evoked contractions of guinea-pig ileum.

Thioperamide derivatives

Thioperamide (**170**) (table 19), is the first potent and selective H₃ receptor antagonist.¹⁰ Replacement of the cyclohexyl group of thioperamide by other aliphatic moieties is allowed (see **171**).⁵² However, replacement by aromatic groups results in significant decrease in activity.⁵³ Furthermore, it has been revealed by Windhorst and co-workers that the activity of halogen substituted benzyl analogues depends on the position of substitution and on the specific halogen (**173-184**).⁵⁴ Thus, ortho substitution is “better” than meta or para substitution (*e.g.*, compare **182**, **183** and **184**). Such effects are not observed in other series of H₃ antagonists. Compounds **173-184** were used in a QSAR study,⁵⁴ the results of which support the modelling studies that are described in chapters 4 and 8. As the SAR concerning the lipophilic terminus for the thioperamide series is clearly different from the SAR of other classes of antagonists, several authors have suggested that thioperamide derivatives bind in a different, unique manner to the receptor.^{33,52,54}

Table 19. H₃ activity of thioperamide and analogues.

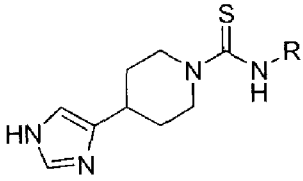
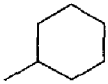

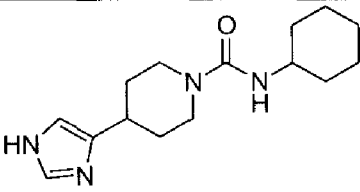
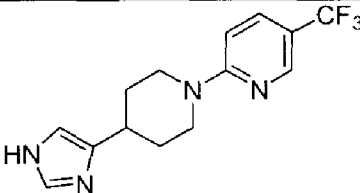
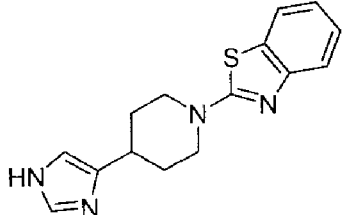
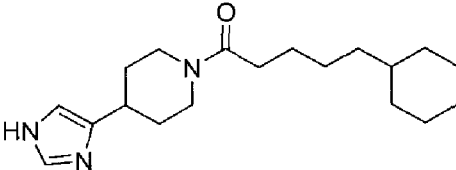
			
No.	compound	R	pA ₂ ^a
170	thioperamide		8.9 ^b pK _i =8.4 ^c
171			9.0
172		benzyl	7.4
173		4-F-benzyl	6.2
174		3-F- benzyl	6.4
175		2-F- benzyl	6.0
176		4-Cl- benzyl	7.2
177		3-Cl- benzyl	7.8
178		2-Cl- benzyl	8.2
179		4-Br- benzyl	6.8
180		3-Br- benzyl	7.6
181		2-Br- benzyl	7.8

Table 19 (continued)			
182		4-I- benzyl	6.7
183		3-I- benzyl	7.6
184		2-I- benzyl	8.2
185			7.3 ^b
186			7.4 ^b
187			6.6 ^c
188			7.4 ^b

^a Electrically evoked contractions of guinea-pig ileum.

^b K⁺-Stimulated [³H]-histamine release from rat cortex.

^c Inhibition of [³H] N^α-methylhistamine binding to rat cortex.

Although it has been revealed that thioperamide (**170**) has a rather good *in vivo* activity (ED₅₀=1.1 mg/kg) and also has blood-brain barrier penetration,^{55,56} the potentially toxic thiourea moiety of thioperamide (**170**) has prohibited clinical use of this selective H₃ antagonist.^{57,58} Hence, replacement of the thiourea moiety by bioisosteric moieties has received substantial attention. Replacement by a less toxic urea moiety results in **185**, which has, however, a significantly lower activity than thioperamide (**170**).³⁸ This reduced activity has been described as “puzzling”,⁵⁹ as thioperamide (**170**) can be considered a ring-closed analogue of **168** (table 14) and an analogous replacement of the thiourea group of compound **168** with a urea group (leading to **169**) does not result in a decrease of activity. The model

that is presented in chapter 8 suggests differences in binding mode for compound **168** and its ring-closed analogue thioperamide (**170**).

Other efforts to replace the thiourea moiety include replacement with an aromatic nitrogen atom-containing heterocycle, leading to compound **186**³³ and **187**.⁶⁰ In addition, the thiourea moiety of thioperamide has been replaced by amide moieties. Finally, compound **188** has a lower *in vitro* activity than thioperamide (**170**), but has improved *in vivo* activity and a better blood-brain barrier penetration.^{61,62,63}

Iodoproxifan and its analogues

Ganellin and co-workers developed a new series of H₃ antagonists (**189**, **190** and **191**) that show high *in vitro* (table 20) as well as excellent *in vivo* activity (ED₅₀ of 0.5, 4.9 and 0.6 mg/kg, respectively).³⁴ This series lacks a basic group in the imidazole side chain. Instead, an oxygen atom is used as polar group. This oxygen atom is connected to the imidazole ring by a spacer that consists of three methylene units.

Table 20. H₃ activity of iodoproxifan and analogues.

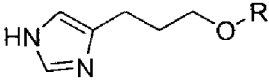
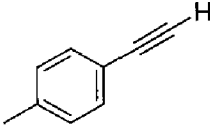
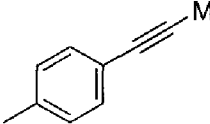
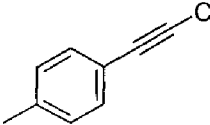
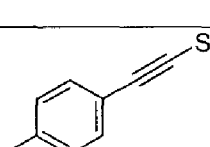
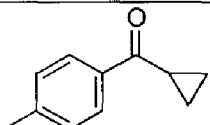
			
No	compound	R	pA ₂ ^a
189		4-CN-phenyl	7.9
190		4-F-phenyl	8.0
191		4-CF ₃ -phenyl	7.8
192		cyclohexyl-CH ₂ -	8.0
193		4-I-phenyl	7.6
194		3-I-phenyl	7.8
195	proxifan	benzyl	7.7
196		4-F-benzyl	8.0
197		3-F-benzyl	7.8
198		4-Cl-benzyl	7.7
199		4-Br-benzyl	7.8
200	iodoproxyfan	4-I-benzyl	8.3
200	[¹²⁵ I]iodoproxyfan	4-[¹²⁵ I]-benzyl	pK _i =10.2 ^b
201		3-I-benzyl	8.2
202		4-Ph-Ph	6.9
203		1-naphthyl	6.8
204		2-naphtyl	6.6

Table 20 (continued)			
205		(Ph) ₂ -CH-	7.6
206		(4-F-Ph) ₂ -CH-	7.6
207		Ph-CH ₂	7.7
208		Ph-(CH ₂) ₂	7.6
209		Ph-(CH ₂) ₃	7.8
210		Ph-(CH ₂) ₄	7.0
211	FUB 181	4-Cl- Ph-(CH ₂) ₃	7.9
212			8.6
213			8.1
214			6.8
215			7.4
216	ciproxifan		9.3

^a K⁺-Stimulated [³H]-histamine release from rat cortex.

^b Displacement of [³H]N^α-methylhistamine from rat cortex.

At the same time as Ganellin's disclosure, Schunack and co-workers reported similar H₃ ligands. Exploiting the synthetical feasibility of this class and using the readily available intermediate compound 3-(1*H*-imidazol-4-yl)propanol, a large series of derivatives have been prepared, varying in the lipophilic tail that is attached to the oxygen atom. A selection of these compounds is presented in table 20.

Introduction of a cycloalkyl group (**192**) or aromatic moiety on the oxygen atom leads to potent compounds. The position of halogenation on the aromatic ring does not have a

significant effect on the activity (compare **200** and **201**).⁶⁴ The compound iodoproxyfan (**200**) has a high H₃ receptor affinity (although on functional assays, the compound is, surprisingly, not very active). Iodoproxyfan has been [¹²⁵I]-labelled and used to radio-tag the H₃ antagonistic binding site.^{65,66,67} However, it was later disclosed that this compound behaves as a partial agonist in mouse brain cortex and guinea-pig ileum.^{68,69,70} This agonistic activity of iodoproxyfan (**200**) is somewhat surprising, as the compound lacks a basic group in the heterocycle side chain that was thought to be essential for stimulating a biogenic amine receptor. Introducing bigger aromatic moieties leads to decreased H₃ antagonistic activity (**202-206**), indicating that the hydrophobic pocket of the receptor cannot accommodate substituents that are too large.⁷¹ However, these compounds, being more lipophilic, have an excellent *in vivo* activity (e.g., ED₅₀= 1.1 mg/kg for **206**). Recently, the *in vivo* activity of this series was further optimized. By varying the length of the alkylene spacer between the oxygen atom and the terminal phenyl group, it was revealed that a propylene spacer is ideal for *in vivo* activity.⁷² Substitution of the phenyl group with a chloro atom led to FUB181 (**211**), a very potent ligand *in vivo* (ED₅₀=0.8 mg/kg). More recently, the alkynylphenyl derivatives **212-215** have been described.⁷³ Compound **212** has the highest *in vitro* and *in vivo* activity (ED₅₀=0.12 mg/kg). Bigger substituents of the alkyne moiety reduced both the *in vitro* and *in vivo* activity.

Ligand **215** has been described as the first compound containing a silyl moiety which shows H₃ activity. The latest modifications have led to ciproxifan (**216**), a compound with a high *in vitro* and *in vivo* activity (ED₅₀=0.14 mg/kg).⁷⁴ Furthermore, ciproxifan (**216**) is a selective H₃ ligand with good oral bio-availability; it may therefore become an important pharmacological tool and probably a promising compound for further clinical development.

H₃ antagonists containing ester and carbamate moieties

Applying the synthetic intermediate used for synthesizing iodoproxyfan analogues, a series of carbonylic analogues have been developed. A number of the compounds are shown in table 21. Ester derivatives like **217-221** are moderately active.⁶⁶ Carbamate derivatives (**222-238**) have been prepared as well; several of these compounds have a high activity, both *in vitro* and *in vivo*.⁷⁵ The most potent compounds *in vivo* are **223** and **224** (ED₅₀=1.3 mg/kg for both compounds). As has been shown for the iodoproxyfan series, introduction of bulky groups as a lipophilic terminus within these series is not allowed for H₃ activity (see **228**). Recently, a series of chiral N-alkyl chain carbamates has been reported, but no stereospecificity was observed (compare **237** and **238**) on the H₃ receptor.⁷⁶

Table 21. Histamine H₃ receptor activity of a series of carbamates.

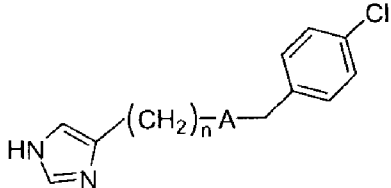
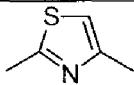
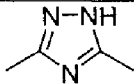
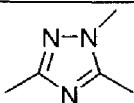
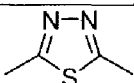
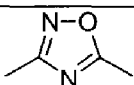
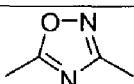
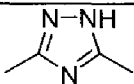
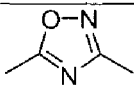
No.	X	R	pA ₂ ^a
217	-	4-I-phenyl	7.7
218	-	3-I-phenyl	7.6
219	-	2-I-phenyl	7.4
220	-	4-I-Ph-CH ₂	7.8
221	-	Ph-(CH ₂) ₂	7.3
222	NH	cyclohexyl	7.2
223	NH	phenyl	7.8
224	NH	4-F-phenyl	7.6
225	NH	4-Cl-phenyl	8.4
226	NH	4-Br-phenyl	7.7
227	NH	4-I-phenyl	8.0
228	NH	2-naphthyl	6.9
229	NH	Ph-(CH ₂)	8.0
230	NH	4-F-Ph-(CH ₂)	7.6
231	NH	4-Cl-Ph-(CH ₂)	7.8
232	NH	4-I-Ph-(CH ₂)	7.9
233	NH	cyclopentyl-(CH ₂)	7.5
234	NH	cyclohexyl-(CH ₂)	8.0
235	NH	Ph-(CH ₂) ₂	8.0
236	NH	Ph-(CH ₂) ₃	7.6
237	NH	(R)-CH ₃ -CH ₂ (CH ₃)CH	7.7
238	NH	(S)-CH ₃ -CH ₂ (CH ₃)CH	7.6

^a K⁺-Stimulated [³H]-histamine release from rat cortex.

Replacement of the polar groups in the imidazole side chain by heterocycles

It has been shown that the polar group in the side chain may be replaced by an aromatic five membered heterocycle while keeping H_3 affinity (table 22).⁷⁷ The propylene linker was found to be optimal for activity of the triazole analogues (compare **240** and **245**), while the ethylene linker seemed better for the oxidiazole series (compare **244** and **246**). Compound **243** has an excellent *in vivo* activity via the oral route ($ED_{50}=1.2$).

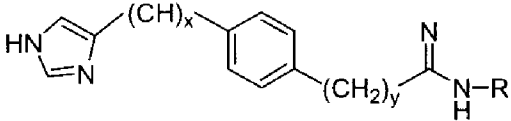
Table 22. H_3 affinity of ligands that have a heterocycle-containing linker.

			
No.	n	A	pK_i^a
239	2		6.7
240	2		6.7
241	2		6.9
242	2		7.0
243	2		7.7
244	2		8.2
245	3		7.9
246	3		7.6

^a Displacement of [3H]N ^{α} -methylhistamine on rat cortex.

Recently, a novel class of antagonists has been described.⁷⁸ The compounds, presented in table 23, have a phenyl ring-containing linker connecting the imidazole moiety with a basic amidine group. A lipophilic terminus is attached to the amidine group. It seems that the central phenyl ring is best connected to the imidazole group by a methylene unit (compare **247** and **252**). A methylene unit is also ideal for connecting the central phenyl ring with the amidine unit (compare **248**, **253** and **254**). No further derivatives of the compound with the optimised linker (*i.e.*, analogues of **253** that have different lipophilic tails) have yet been reported.

Table 23. H₃ affinity of ligands that have a phenyl ring-containing linker.

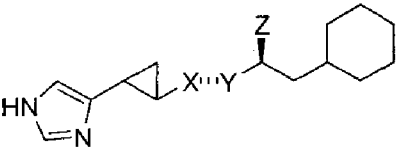
				
No.	X	Y	R	pK _i ^a
247	1	0	H	7.4
248	1	0	4-Cl-Ph-CH ₂	8.0
249	1	0	4-CH ₃ O-Ph-CH ₂	6.0
250	1	0	4-CF ₃ -Ph-CH ₂	6.8
251	1	0	Ph-(CH ₂) ₂	6.4
252	2	0	H	6.8
253	1	1	4-Cl-Ph-CH ₂	8.1
254	1	2	4-Cl-Ph-CH ₂	6.8

^a Displacement of [³H] N^α-methylhistamine from guinea-pig brain tissue.

Cyclopropane and acetylene-containing H₃ antagonists.

Philips and co-workers have developed highly potent H₃ antagonists that incorporate the side chain in a cyclopropane ring (table 24 and 25).⁷⁹ Their studies have revealed that a *trans*-(1R, 2R)-cyclopropane ring fixes the side chain of antagonists in an ideal conformation for binding to the receptor. The compounds are the first antagonists showing enantiospecificity on the H₃ receptor (compare **255** and **256**, and also **259** and **261**). Replacing the amide moiety by a hydrophobic olefin bond results in compounds with even higher affinity, provided the double bond is in a *trans*-configuration (compare **255** and **257**). An olefin in the *cis*-configuration drastically reduces H₃ affinity (**258**). The basic amino group in the side chain seems to be involved in binding to a hydrogen-bonding acceptor site point of the receptor (compare **257** and **259**) but was nevertheless sacrificed to improve the blood-brain barrier penetration capacity.

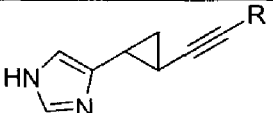
Table 24. H₃ affinity of cyclopropane-containing antagonists.

				
No.	Cyclopropane configuration	X ^{••} Y	Z	pK _i ^a
255	1R, 2R	NH-CO	NH ₂	8.7
256	1S, 2S	NH-CO	NH ₂	7.7
257	1R, 2R	C=C (<i>E</i>)	NH ₂	9.4
258	1R, 2R	C=C (<i>Z</i>)	NH ₂	7.0
259	1R, 2R	C=C (<i>E</i>)	H	8.6
260	1R, 2R	C=C (<i>Z</i>)	H	8.2
261	1S, 2S	C=C (<i>E</i>)	H	7.2
262	1S, 2S	C=C (<i>Z</i>)	H	7.0

^a Displacement of [³H] N^α-methylhistamine from rat cortex.

Subsequent replacement of the olefin bond by an acetylene moiety results in an extremely potent class of H₃ antagonists.⁸⁰ Aliphatic groups seem to be ideal as the lipophilic terminus. Resolution of the most active racemic mixture **268** in this series, gave the enantiopure **269** and **270**, the former being the most potent H₃ antagonist to date.

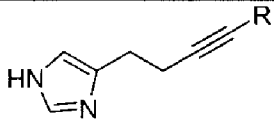
Table 25. H₃ affinity of more cyclopropane-containing antagonists.

			
No.	cyclopropane configuration	R	pK _i ^a
263	(±)	(CH ₂) ₂ CH ₃	8.3
264	(±)	(CH ₂) ₂ CH(CH ₃) ₂	8.2
265	(±)	(CH ₂) ₂ - <i>cyclo</i> -C ₃ H ₉	8.8
266	(±)	(CH ₂) ₂ - <i>cyclo</i> -C ₆ H ₁₁	8.7
267	(±)	(CH ₂) ₄ C ₆ H ₅	8.0
268	(±)	(CH ₂) ₂ - <i>tert</i> -butyl	9.5
269 GT2331	(1R, 2R)	(CH ₂) ₂ - <i>tert</i> -butyl	9.9
270	(1S, 2S)	(CH ₂) ₂ - <i>tert</i> -butyl	8.3

^a Displacement of [³H] N^α-methylhistamine from rat cortex.

Replacing the cyclopropane ring with a flexible ethylene spacer, thus removing the chiral centers, leads to reduced H₃ activity. However, these compounds seem more suitable for development as drug candidates (table 26). It has been reported that GT-2286 (**275**) crosses the blood-brain barrier very efficiently and has a ED₅₀ between 0.3-1.0 mg/kg (orally).

Table 26. H₃ affinity of flexible, acetylene-containing antagonists.

		
No.	R	pK _i ^a
271	H	7.2
272	CH ₂ CH ₃	7.1
273	(CH ₂) ₂ CH ₃	7.6
274	(CH ₂) ₂ CH(CH ₃) ₂	8.4
275	(CH ₂) ₂ - <i>cyclo</i> -C ₅ H ₉	9.0
276	(CH ₂) ₂ - <i>cyclo</i> -C ₆ H ₁₁	8.5
277	(CH ₂) ₄ C ₆ H ₅	8.4
278	(CH ₂) ₂ - <i>tert</i> -butyl	9.1
279	(CH ₂) ₄ CH ₃	8.2
280	(CH ₂) ₆ CH ₃	8.6

^a Displacement of [³H] N^α-methylhistamine from rat cortex.

1.2.3 Histamine H₃ receptor partial agonists.

Some compounds with an intrinsic activity of $0 < \alpha < 1$ have been mentioned in the previous subsections. Recently, it has been revealed that more compounds that were initially described as antagonists are actually (partial) agonists (*vide infra*). It seems likely that more thorough testing of the compounds presented in the previous subsections will identify more partial agonists. Partial agonism on the H₃ receptor is gaining interest and very recently, several groups have described more systematic studies to gain further insight in the factors that determine efficacy on the H₃ receptor.

Kovalainen *et al.*, designed hybrids of the agonist R(α)-methylhistamine (**12**) and the "antagonist" iodoproxyfan (**200**).⁸¹ The H₃ pharmacological data of their compounds are difficult to interpret but intriguing (table 27). All hybrids are less active than their (α)-methylhistamine or iodoproxyfan analogues (see table 3 and table 20, respectively). Nevertheless, it can be seen that the receptor still prefers the stereochemistry of the amino group that is also found in the potent agonist (R)- α -methylhistamine (**12**); for these hybrids that is the (S)-enantiomer (compare **181** and **182**). The stereospecificity of the ligands with reduced affinity is low (Pfeiffer's rule).⁸² It seems that bigger lipophilic tails hamper binding to the receptor (compare **282**, **284**, **286** and **288**).

Table 27. H₃ affinity and efficacy of some iodoproxyfan/(R)- α -methylhistamine hybrids.

No.	configuration n	R	pK _i ^a	agonistic response ^b	antagonistic response ^b
281	(R)	cyclohexyl-CH ₂	6.8	+	-
282	(S)	cyclohexyl-CH ₂	7.9	+	-
283	(R)	Ph-CH ₂	6.5	-	-
284	(S)	Ph-CH ₂	7.3	±	-
285	(R)	4-Br-Ph-CH ₂	6.7	±	+
286	(S)	4-Br-Ph-CH ₂	7.1	±	+
287	(R)	4-I-Ph-CH ₂	6.7	-	+
288	(S)	4-I-Ph-CH ₂	6.6	-	+

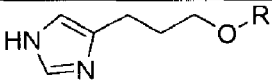
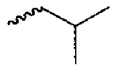
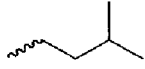
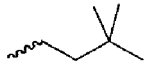
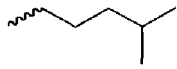
^a Displacement of [³H] R-(α)-methylhistamine on rat cortex.

^b H₃-Receptor dependent activation of G-proteins with GTP μ [³⁵S] autoradiography of rat brain tissues.

The functional activities of these compounds were determined on a qualitative assay that cannot distinguish between full and partial agonistic activity, nor can determine quantitative differences between the compounds. The higher affinity compounds **281** and **282** show clear agonistic activity, ligands **284-286** have some agonistic activity, but no agonism can be detected for **287** and **288**. Antagonistic activity can only be detected for **285-288**. Surprisingly, compound **283** shows neither agonistic nor antagonistic activity. The data are puzzling and no straightforward rationale can be given. A ligand that combines structural features of both agonists and antagonists might induce a conformation of the receptor that is an intermediate of the active and inactive state. Unfortunately, the activation mechanism of G-protein-coupled receptors (GPCRs) is not understood. In this light, ligands such as those presented in table 27 are interesting, as they can help, in combination with molecular modelling studies, to unravel the molecular basis of receptor stimulation.

Very recently, branched O-alkyl analogues of the iodoproxyfan class have been described (table 28).⁸³ These compounds reveal that small changes can have drastic effects on the efficacy. Thus, replacing the O-alkyl substituent from a 2-methylpropyl to a 4-methylbutyl group (compare **289** and **290**) leads to a transition from a moderate affinity antagonist to a higher affinity partial agonist. Introduction of an extra branch by using a 3,3-dimethylbutyl chain (compare **290** and **291**) results in a compound with similar affinity but with a significantly higher efficacy. Even more surprising was the finding that two compounds (**290** and **291**) were full agonists under *in vivo* conditions. Again, this study shows that the transition from antagonist to agonist responses is caused by only small structural changes.

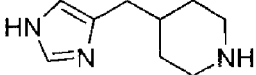
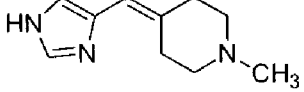
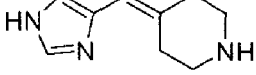
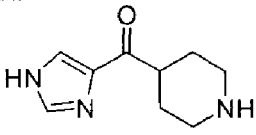
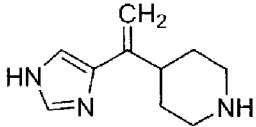
Table 28. H₃ activity and efficacy of some iodoproxyfan analogues.

			
No.	R	pA ₂ ^a	Efficacy α ^a
289		6.8	-
290		7.8	~0.22
291		8.0	0.55
292		8.0	~ 0.25

^a K⁺-Stimulated [³H]-histamine release on rat cortex.

Certain structural modifications of the agonist immepip (**54**) also lead to compounds with reduced efficacy. It has been reported in patent literature that the rigid analogue (**293**) has a moderate H_3 affinity.⁸⁴ It has been suggested that the compound is an agonist, as it is structurally related to immepip.^{85,86} However, when incorporating the structure of **293** in the pharmacophore model described in chapter 4, it fitted the antagonistic, rather than the agonistic binding site. Synthesis of the analogues **294-296** and subsequent pharmacological testing has revealed that compounds **294** and **296** are, in fact, partial agonists and that analogue **295** has only a very low antagonistic activity. For a detailed discussion, including the molecular modelling studies using these ligands, the reader is referred to chapter 6.

Table 29. Histamine H_3 receptor activity of immepip and the derivatives.

No.	Compound	Structure	pA_2^a	Efficacy α^a
54	immepip		$pD_2=8.0$	1
293			$pK_i=7.9^b$	- ^c
294			7.4	0.5
295			5.3	0
296			7.4	0.5

^a Electrically evoked contractions of guinea-pig ileum.

^b Displacement of [3H] R-(α)-methylhistamine on rat cortex.

^c Not determined.⁸⁴

1.2.4 Non-imidazole histamine H₃ receptor ligands.

For a long time, it was believed that the imidazole ring was essential for H₃ activity. Only very recently, non-imidazole antagonists have been described. Menge and co-workers have disclosed potent 4-aminopiperidine derivatives, *e.g.*, **297** and **299** ($pA_2=7.81$ and $pA_2=7.41$, respectively; electrically evoked contractions of guinea-pig ileum).^{87,88} Ganellin *et al.* have published series of N-[5-(4-substituted phenoxy)pentyl] pyrrolidines (Figure 1).⁸⁹ The best compounds, **298** and **300**, have a high *in vitro* activity ($pA_2=7.7$; K⁺-stimulated [³H]-histamine release from rat cortex), but also reveal a high *in vivo* activity (orally), with ED₅₀ about 1 mg/kg. It can be anticipated that, in the near future, more non-imidazole compounds will be found. Subsequent SAR studies might reveal whether these compounds interact at the same binding site of the H₃ receptor as the imidazole-containing ligands.

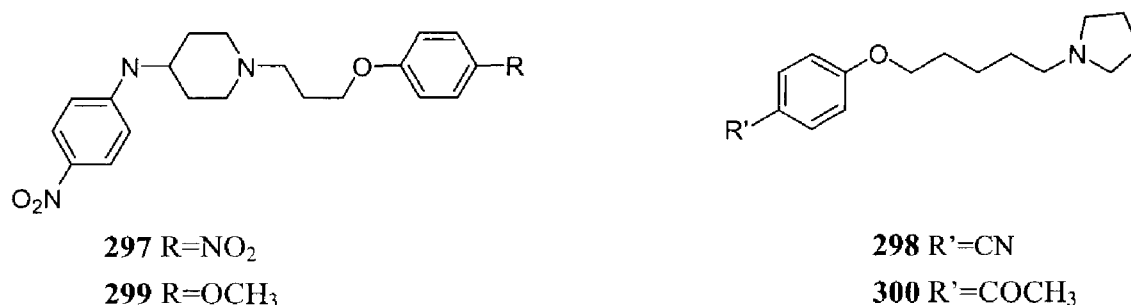


Figure 1. Non-imidazole H₃ receptor ligands.

1.3 Molecular modelling studies of histamine H₃ receptor ligands.

Molecular modelling studies represent a challenging aspect of medicinal chemistry, as they facilitate drug design and lead optimisation processes. Furthermore, such studies may contribute to the unravelling of molecular mechanisms involved in receptor stimulation. Until very recently, the gene encoding the histamine H₃ receptor had not been cloned and hence, virtually nothing is yet known about the receptor topography. Therefore, modelling studies regarding the histamine H₃ receptor have been limited to receptor mapping, making use of the properties of the ligands. In this subsection, a review will be given of the existing histamine H₃ ligand-binding models and complying views on receptor activation mechanisms that evolved from modelling studies.

Tautomerism of imidazole-containing H₃ ligands

There is general consensus on the fact that the endogenous agonist histamine, 2-(imidazole-4-yl)ethylamine, binds to the receptor in its monocationic state (protonated at the side chain amine group). The monocationic form is predominantly (96.6%) present at pH 7.4.⁹⁰ In this state, the neutral imidazole ring can exist in two different tautomeric forms, denominated by proximal (π) and tele (τ), respectively (Figure 2).

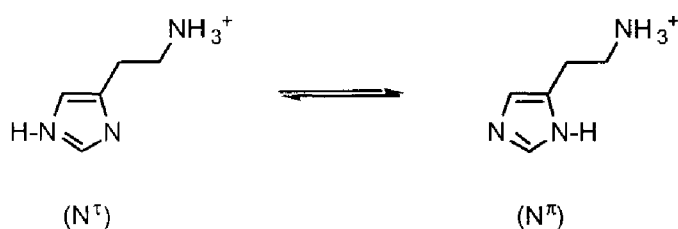


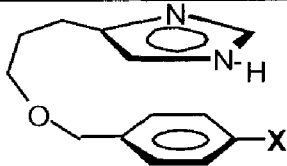
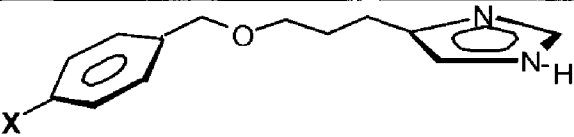
Figure 2. The two tautomeric forms of the monocation of histamine.

For the histamine H₂ receptor, the development of potent histamine analogues that have functional groups replacing the imidazole heterocycle^{13,91} in combination with molecular modelling studies⁹² have revealed that the N^{π} tautomer of histamine is the biologically active form for this subtype receptor.⁹³ For the histamine H₃ receptor, information regarding the bioactive tautomer of the ligand is scarce as alteration of the imidazole is, for agonists, not tolerated. Whereas substituents on the 4(5)-imidazole ring lead to dramatically reduced H₃ agonistic activity, replacement of the imidazole by other groups results in compounds with no H₃ agonistic activity at all. At present, all published, highly potent H₃ receptor antagonists possess an imidazole moiety and, as found for agonists, substitution or alteration of this

heterocycle gives rise to severely reduced H₃ activity. We therefore claim that imidazole binding to the histamine H₃ receptor is compulsory for both agonists and antagonists and two hydrogen bonds between the imidazole moiety and the receptor can be anticipated. However, no definite conclusions can be drawn concerning the specific tautomer that is recognised by the H₃ receptor. The newly discovered non-imidazole antagonists (see subsection 1.2.4) bind, most likely, in another way to the receptor than the imidazole-containing ligands.

It has been suggested that the unique tautomeric property of the imidazole ring is essential for efficacy at the histamine H₃ receptor.⁶⁹ As described in the previous subsection, thorough pharmacological testing of iodoproxifan and analogues revealed partial agonistic activity of these compound.⁷⁰ Low *et al.* suggested a correlation between efficacy at H₃ receptors and the tendency to adopt folded conformations of these compounds (table 30).⁶⁹ The authors postulate that in the folded conformation the presence of intramolecular π -stacking between the imidazole and the aromatic ring in the side chain of the ligands hampers imidazole tautomerisation and thereby may reduce agonistic efficacy.

Table 30. Correlation between the folding and the efficacy (% α) expressed at H₃ receptors.⁶⁹

				
folded conformation		open conformation		
No.	Compound	X	% folded conformations	% α^a
195	proxifan	H	100	33
196		F	85	15
198		Cl	56	58
199		Br	26	84
200	iodoproxifan	I	33	82

^aRelative to R- α -methylhistamine.

The bioactive conformation of agonists

Histamine (**1**) is a flexible molecule that can adopt numerous conformations (figure 3). These can be described in terms of three torsional angles τ_1 , τ_2 and τ_3 . Quantum mechanical calculations on conformations for histamine^{94,95} reveal that with respect to τ_2 , the histamine monocation is found basically in the *trans* and *gauche* conformation ($\tau_2=180^\circ$ or 60°). The values for τ_1 and τ_3 are less restricted.

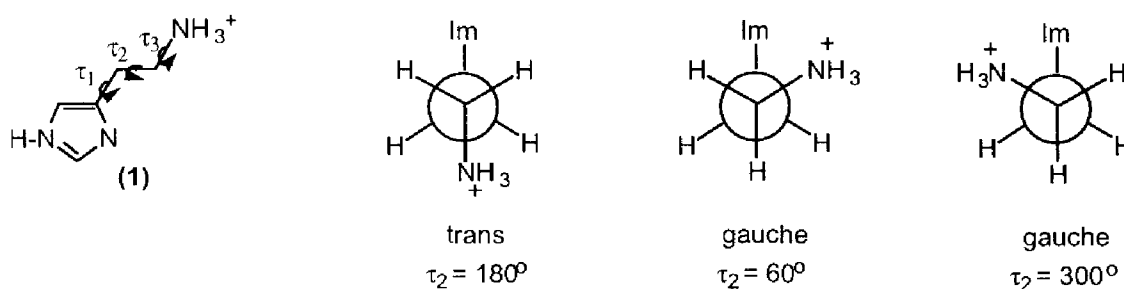


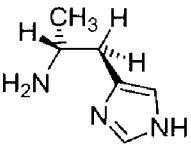
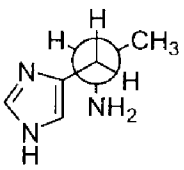
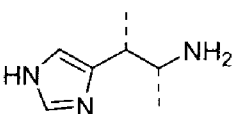
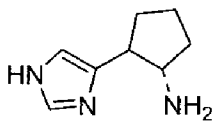
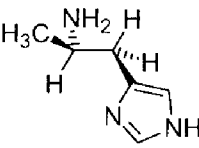
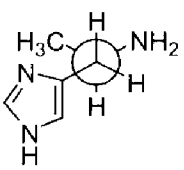
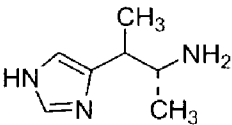
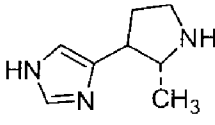
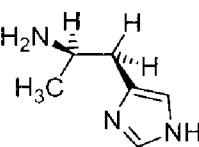
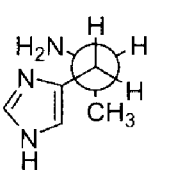
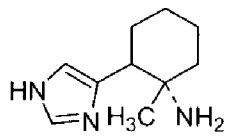
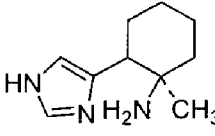
Figure 3. The conformations of histamine (**1**) described by 3 torsion angles (τ_1 , τ_2 and τ_3 , respectively). Torsion angle τ_2 is found fundamentally in the *trans* and *gauche* conformations.

Conformational analysis of the standard H_3 agonist (R)- α -methylhistamine (**12**) using molecular mechanics leads to a similar result as found for histamine.²² (R)- α -methylhistamine (**12**) is predominantly found in three conformations (neglecting the rotational freedom of the τ_1 axis, cf. Figure 3). Only minor energetic differences (<1.5 kcal/mol) were found between these three rotameric conformations. Based on these conformational studies, Shih and co-workers designed and synthesised a series of conformationally constrained H_3 agonists to investigate the bioactive conformation and to explore the sterical requirements of H_3 receptor agonists (table 31).²² Each of the three aforementioned conformations was mimicked by two conformationally restricted analogues of histamine. These results have been depicted in table 2: compound **16** and **32** aim at mimicking the *gauche* conformer I; both the racemic mixture (**14-15**) and the racemic mixture (**35-36**) should mimic the *anti* conformer II; whereas the *trans* and *cis* isomers of **39** should mimic the *gauche* conformer III.

Shih *et al.* determined the H_3 activity in terms of H_3 binding (table 31). The pharmacological data show that the racemic mixtures (**14-15**) and (**35-36**) are the most potent H_3 ligands in this series. Therefore, it can be concluded that the bioactive conformation of the studied compound (R)- α -methylhistamine (**12**) is the *anti*-conformer II. However, it should be noted that the activity of these compounds may also be influenced by steric factors, especially as

large substituents on the ethylene chain of histamine are not allowed for agonistic activity (see previous subsections).

Table 31. The design of conformationally restricted H₃ agonists by Shih and co-workers.²²

Conformers of (R)- α -methylhistamine		Compounds synthesised	
structure	newman projection formula		
I (gauche) 		 (±)- 16 $pK_i=8.0$	 (±)- 32 $pK_i=6.3$
II (anti) 		 (±)-(14-15) $pK_i=9.1$	 (±)-(35-36) $pK_i=8.1$
III (gauche) 		 (±)- <i>trans</i> - 39 $pK_i<5.7$	 (±)- <i>cis</i> - 39 $pK_i<5.7$

^a For all compounds, the racemic mixtures are tested. For some compounds, the pharmacological data of the enantiopure compounds are also available (*e.g.*, **14** and **15**). See subsection 1.2.1.

^b Displacement of [³H] N ^{α} -methylhistamine from guinea-pig brain tissue.

Contrary to the finding of Shih and co-workers, Mazurek *et al.* claimed, on the basis of *ab initio* molecular orbital calculations, that the bio-active conformation of (R) α -methylhistamine (**12**) has an intramolecular hydrogen bond between the cationic side chain amine and the basic N π atom of the imidazole.⁹⁶ This conformation corresponds to one of the

gauche-conformers depicted in figure 3 and table 31. Recently, Kovalainen *et al.* described a ^1H NMR study using H_3 agonists that showed that 52%-61% of the molecules exist in this conformation.⁹⁷ To test whether this folded conformation is biologically active, we synthesised (S) α ,(S) β -*trans*-cyclopropylhistamine (VUF 5297; **30**) which exhibits H_3 agonistic activity (for details see chapter 4). This rigid cyclopropyl-containing histamine analogue is unable to form an intramolecular hydrogen bond and its activity therefore reveals that internal hydrogen bond formation is not essential for H_3 activity. This conclusion is in line with the aforementioned model by Low and co-workers, as intramolecular hydrogen bonding would hamper a tautomeric shift of the imidazole, which is suggested to be essential for histamine H_3 receptor activity.

Sippl and co-workers were the first to develop a pharmacophore model for histamine H_3 agonists.⁹⁸ Superimposing all relevant conformations of a series of selected agonists (shown in figure 4) and using (R) α ,(S) β -dimethylhistamine (**14**) as a template, two pharmacophores evolved in which all imidazole rings and all protonated side chain nitrogen atoms could be perfectly superimposed. Only one pharmacophore revealed a good overlap of the hydrophobic part of the side chains and was therefore selected for further investigation. Histamine reveals a *gauche-trans* conformation in this model (*cf.* Figure 3, τ_1 and τ_2). The methyl group of Sch 49648 (**36**) occupies the same region of space as the (R) α -methyl group of (R) α ,(S) β -dimethylhistamine (**14**) while the pyrrolidine ring overlaps with the (S) β -methyl group. The relatively longer side chains of imetit (**41**) and SKF 91606 (**44**) adopt folded conformations, thereby occupying the same region of space as the (S) β -methyl group of (R) α ,(S) β -dimethylhistamine (**14**). It was noted that of the different stereoisomers of cyclopropylhistamine, the (S) α ,(S) β -enantiomer (**30**) fits best in the derived pharmacophore. Recent studies in our laboratories indeed established (S) α ,(S) β -cyclopropylhistamine (VUF 5297, **30**) as the eutomer of this small and rigid compound (chapter 4), validating the theoretical findings. For a graphical illustration of the H_3 agonistic pharmacophore the reader is referred to chapter 4.

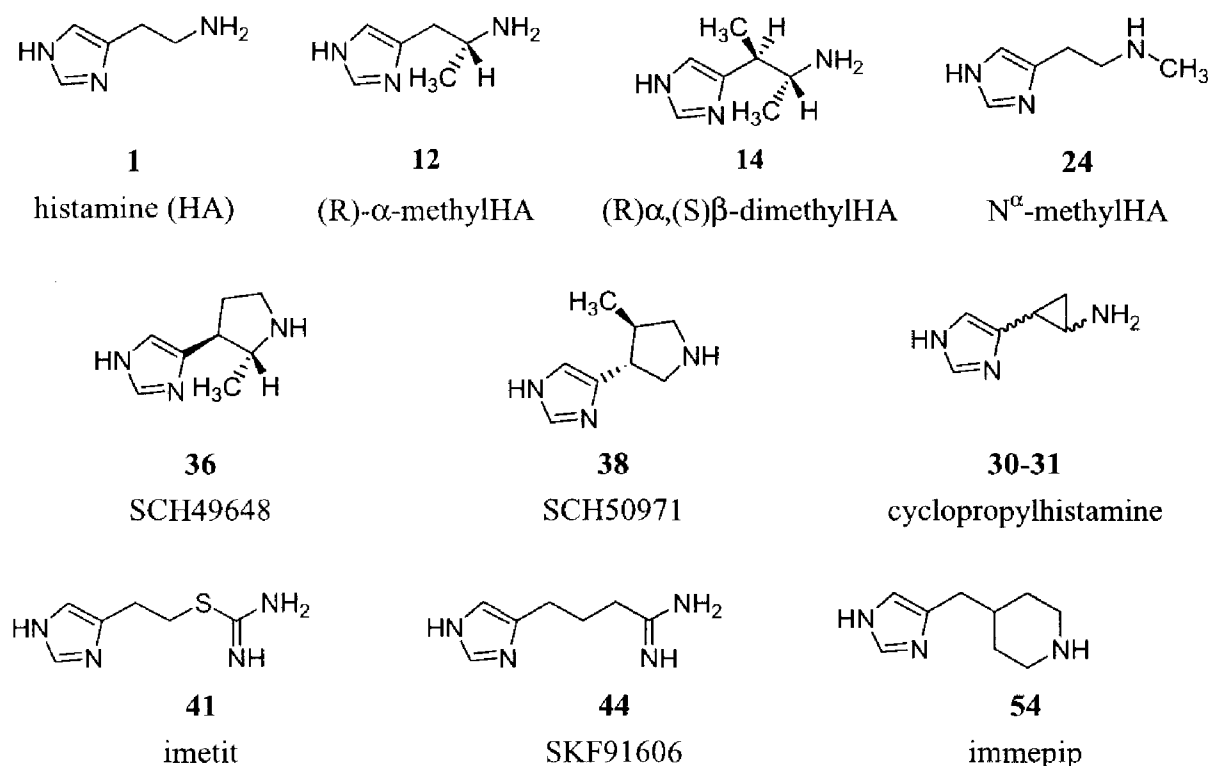


Figure 4. H₃ receptor agonists investigated by Sippl *et al.* ^aK⁺-Stimulated [³H]-histamine release from rat cortex ^bElectrically evoked contractions of guinea-pig ileum ^cThe authors had no information about the H₃ activity of the distinct stereoisomers. See text for details.

Subsequently, and using their H₃ agonistic pharmacophore as a template, Höltje and Sippl built a pseudoreceptor model for the H₃ receptor agonist binding site.⁹⁹ The pseudoreceptor concept seeks to construct a model receptor that mimics the essential ligand-macromolecule interactions of the true biological receptor. Starting point for the construction of a pseudoreceptor using the receptor-mapping program YAK^{100,101} are the superimposed ligands in their bioactive conformation. Potential binding sites (anchor points) are defined for each of the ligand molecules of the pharmacophore. For this purpose the program YAK uses an extensive database that holds information about ligand-receptor interactions. Suitable binding partners (*e.g.*, amino-acids, metal ions and solvent) result from this process and are positioned in three-dimensional space. The collection of binding partners constitutes a pseudoreceptor for the ligands used as the original template.

Höltje and Sippl selected a representative “training set” of twelve superimposed structures. The aforementioned YAK process resulted in a pseudoreceptor that consists of six amino-acid residues. In this model, the imidazole ring of the ligands is bound to a tyrosine residue that donates a proton to the imidazole and an asparagine residue that accepts a proton from the imidazole. In addition to the hydrogen-bonding interactions, the imidazole heterocycle has a hydrophobic interaction with a phenylalanine of the pseudoreceptor. Hence, two distinct H-

bonds and an aromatic interaction of the imidazole ring with the site have been found, which explain the strict binding mode of the imidazole. Around the hydrophobic part of the side chains a leucine and an isoleucine fragment are located. The positively charged side chain nitrogen atoms interact with a negatively charged aspartate of the pseudoreceptor.

The YAK program can be used to estimate relative free energies of binding between ligands and pseudoreceptor. Höltje and Sippl calculated for the training set, used to construct the pseudoreceptor, that the correlation coefficient for experimental *versus* calculated free energies of binding equals 0.99 and the RMS deviation is 0.21 kcal/mol.^{65,102} Subsequently, Höltje and Sippl tested the pseudoreceptor model by predicting biological binding data for a test set of four additional agonists not included in the construction of the pseudoreceptor training set. The test set revealed a RMS deviation of 0.66 kcal/mol, indicating the high accuracy of the model. It has to be appreciated, however, that structural diversity between the compounds used to construct the model and the test set is rather limited. More severe testing of the predictive power of the pseudoreceptor with structurally more diverse agonists like immezip (**54**) has not been reported.

Bioactive conformation of H₃ agonists and antagonists

In chapter 4 of this thesis, a qualitative H₃ ligand-binding model is described that reveals the putative interaction of both H₃ agonists and antagonists with an aspartate residue of the receptor. The model reveals a molecular determinant explaining efficacy as the conformation of the aspartic-acid residue differs according to whether it is binding to agonists or antagonists. The agonists in this study reveal an identical biologically active conformation as in the model of Sippl *et al.* (*vide supra*). In addition to the binding mode of agonists, the model in chapter 4 describes the bonding mode of antagonists, and it has been revealed that there are two hydrophobic pockets of the H₃ receptor available for binding the lipophilic tails of the antagonists (see chapter 4 for more details).

The derived model has been used by Windhorst and co-workers to gain more insight into the binding properties of one of these lipophilic pockets available for binding antagonists.⁵⁴ A series of thioperamide analogues was synthesised to determine the QSAR of the lipophilic tail for this class of antagonists. As indicated by the results presented in table 32, the activity of halogen substituted benzyl analogues depends on the position of substitution and on the given halogen. Substitution at the ortho position leads to the most potent derivatives, except for fluorine for which no significant effect is observed.

Table 32. pA₂ values^a of several halogen-substituted benzyl analogues of thioperamide (**172-184**). See table 19 for more details.

X	ortho	meta	para
H	7.4	7.4	7.4
F	6.0	6.4	6.2
Cl	8.2	7.8	7.2
Br	7.8	7.6	6.8
I	8.2	7.6	6.7

^a Electrically evoked contractions of guinea-pig intestine.

The putative bioactive conformation of thioperamide (**170**), as derived in chapter 4, was used as a (fixed) template. Using this template, the different benzyl substituted analogues (table 32) were constructed. Next, the geometries of these lipophilic tails were optimised using a density functional approach (for details the reader is referred to chapter 4). The energy minimised structures reveal that the dihedral angle (ϕ) between the thiourea moiety and the phenyl group differs depending on the position and nature of substitution of the benzyl ring. This effect is illustrated in Figure 5 by superimposing the three bromo analogues (*cf.* table 32). A good correlation (eq. 1) was found between the antagonistic activity (pA₂) and the dihedral angle ϕ and the charge δ on the substituted carbon atom of the benzyl group ($n=13$, $r=0.93$, $F=31.57$):

$$\text{pA}_2 = -0.02 \phi - 0.933 \delta + 4.72 \quad (\text{eq. 1})$$

The results of this QSAR study support the accuracy of the geometry of the ligand-carboxylate complexes in our qualitative H₃ ligand-binding model described in chapter 4. Furthermore, the findings by Windhorst *et al.* have also been incorporated in the more detailed pharmacophore model for H₃ antagonists that is presented in chapter 8 of this thesis. The latter model was constructed using newly developed molecular modelling tools and describes in more detail the position and shape of the hydrophobic pocket of the receptor.

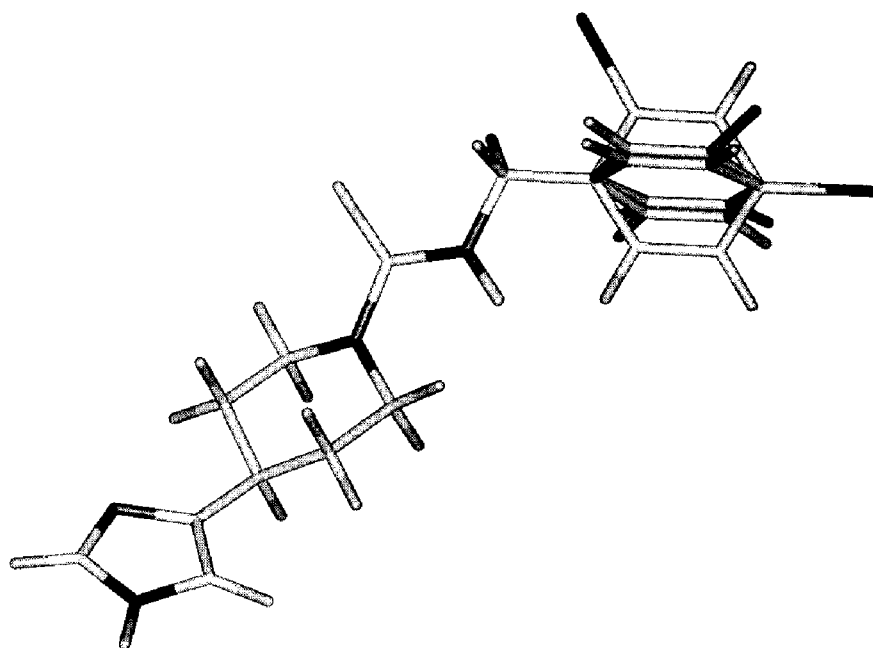


Figure 5. Superposition of the ortho, meta and para-bromo benzyl derivatives **179**, **180** and **181** illustrates the differences in dihedral angle.⁵⁴ See text for more detail.

1. 4 Pharmacological and (patho)physiological aspects of the histamine H₃ receptor.

Over the last decade, considerable progress has been made in understanding the role of the H₃ receptor in pharmacology and (patho)physiology. Arrang and co-workers showed, as described in subsection 1.1, that stimulation of the H₃ receptor down-regulates histamine release.⁹ Subsequently, it was revealed that H₃ receptor stimulation also down-regulates histamine synthesis¹⁰³ (Figure 6). Furthermore, the receptor has been shown (as a heteroreceptor) to modulate the release of other neurotransmitters, *e.g.*, acetylcholine,¹⁰⁴ dopamine,¹⁰⁵ serotonin^{106,107} and noradrenaline.¹⁰⁸ Whereas the H₁ and H₂ receptors are located post-synaptically, the H₃ receptor is found on pre-synaptic neuron endings.¹⁰⁹

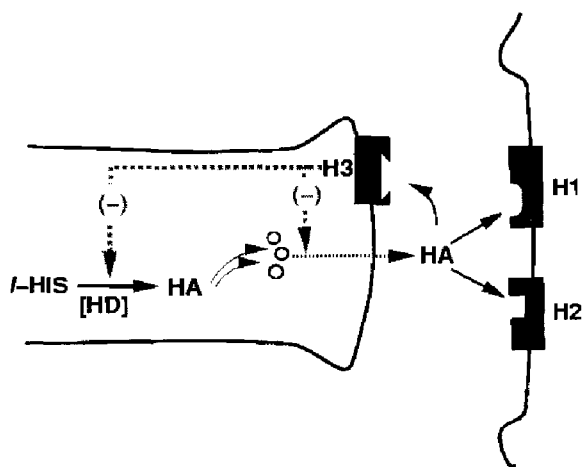


Figure 6. Schematic representation of histaminergic neurotransmission. Abbreviations: *l*-HIS for (*l*)-histidine; [HD] for histidine decarboxylase; HA for histamine. See text for details.

Because inhibitory effects on histamine H₃ receptor-mediated stimuli by G protein toxins (both cholera and pertussin toxin) have been reported,^{110, 111} it was assumed that the histamine H₃ receptor belongs to the superfamily of G protein-coupled receptors, *i.e.* coupled to a G protein of the G_i class.¹¹² Only very recently, the gene encoding for the H₃ receptor has been identified, validating the H₃ receptor as a GPCR. Lovenberg and co-workers expressed the gene of an orphan receptor (GPCR97) that has significant homology to biogenic amine receptors.¹¹³ Transfection of the gene into a variety of cell lines and administration of biogenic amine ligands revealed that selective H₃ agonists provoked an inhibition of forskolin-stimulated cAMP formation. Subsequent analysis revealed a pharmacological profile that matched the histamine H₃ receptor. Comparing the 445-amino-acid sequence of

the H₃ receptor to that of the H₁ and H₂ receptor reveals a low overall homology (20-27%). Most notable was the finding of an aspartic-acid residue in the transmembrane domain III, which is characteristic for the biogenic amine receptor subfamily.^{114,115}

H₃ receptors are well distributed in the peripheral nervous system *e.g.*, in the gastrointestinal tract, the airways and the cardiovascular system, although remarkable species differences have been found, especially in the latter.¹¹⁶ However, the highest density of H₃ receptors is present in the central nervous system. Autoradiography studies with the human brain has revealed that the H₃ receptor is predominantly present in the basal ganglia, hippocampus and cortical areas, *i.e.*, the parts of the brain that are associated with cognition.¹¹⁷ In these areas, the neurotransmitter histamine has, in close association with the other biogenic amines (serotonin, dopamine, noradrenaline, acetylcholine), a role in controlling numerous (patho)physiological processes. The fact that the H₃ receptor plays a central role in regulating the levels of various neurotransmitters (as it is not only an autoreceptor but also a heteroreceptor), makes it an attractive target for drugs for various diseases.

Therapeutic potential of histamine H₃ receptor ligands.

The therapeutic potential of H₃ ligands has not been assessed yet, but various studies have suggested several applications for H₃ ligands, which have been reviewed recently.^{118,119,120} In short, it has been suggested that H₃ agonists might be useful peripherally in the treatment of asthma and diarrhoea (both via reduction of the adrenergic/non-cholinergic as well as cholinergic tonus).^{16,121} Furthermore, these agonists are suggested to reduce microvascular leakage and histamine release from mast cells.^{122,123} Therapeutic use of H₃ receptor antagonists in the periphery remains to be established.

H₃ receptor ligands that are active in the CNS may be used to correct sleep/wakefulness disorders. Histamine has been called a “waking amine”, *i.e.*, histaminergic activity is significantly increased during awake or light periods.¹²⁴ H₃ antagonists are also believed to be able to correct cognitive and memory processes. It has been shown that H₃ antagonists improve the learning rate in rats.¹²⁵ Because H₃ antagonists are also able to normalise motor disturbances, the ligands may be useful in the treatment of the attention-deficit hyperactive disorder (ADHD), a developmental disorder that affects about 5% of the school-age population.¹²⁶ There are some suggestions that the memory enhancing effects of H₃ antagonists might also be useful in the treatment of Alzheimer's disease, especially because histaminergic neurons seem to be largely spared in this neuro-degenerative disease.^{127,128} Histamine H₃ receptors possess a regulatory role in food-intake¹²⁹ and H₃ antagonists may therefore prove useful in treating obesity.¹³⁰ H₃ antagonists have also been shown to decrease

the seizure susceptibility of electrically-induced convulsions in mice, indicating a possible use in treating epilepsy.¹³¹ Furthermore, a role of H₃ antagonists in depression (mainly via the serotonin pathway)¹³² and schizophrenia (through the noradrenaline pathway)¹³³ has been suggested.

Despite all these putative applications, no H₃ ligand has yet been introduced in the clinic. It is believed that the introduction of H₃ receptor-related drugs is seriously hampered by the pharmacokinetic profiles of most (but not all) of the compounds developed so far, *e.g.*, poor blood-brain-barrier penetration,¹³⁴ toxicity¹³⁵ and cytochrome P450 activity.¹³⁶ Only the agonist (R)- α -methylhistamine (**12**) and the antagonist GT2331 (**269**) have entered clinical investigations. Thus, there remains a need for new ligands with improved pharmacokinetic profiles.

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Chapter 2

Scope of this thesis

In chapter 1, it has been indicated that the H_3 receptor is an interesting target for new drugs to treat disorders like Alzheimer's disease, epilepsy, sleep disturbance, obesity and asthma.¹ In 1994, at the start of the studies described in this thesis, many selective H_3 ligands were known. However, clinical use of these compounds as therapeutic agents was prohibited by their poor pharmacokinetic properties, blood-brain-barrier penetration and their interaction with cytochrome P450 isoenzymes.^{2,3} Design of novel histamine H_3 ligands was hampered as there was no thorough understanding of the molecular features of H_3 ligands that are responsible for affinity and efficacy. It was the aim of the research that is described in this thesis to synthesise and test pharmacologically selected compounds that contribute to a better understanding of the various aspects of ligand binding to the H_3 receptor. The new ligands should enable molecular modelling studies that lead to the development of pharmacophore models. In general, these theoretical models cannot be considered "real-life snapshots", as the biological data that forms the basis of the theoretical studies, as well as the computational methods have many limitations. However, when thinking about these pharmacophore models as working hypotheses, they can be appreciated for leading to new insights and generating ideas for new, interesting compounds to synthesise. Thus, the goal was an iterative and synergistic process in which the synthesis, pharmacological evaluation and molecular modelling of H_3 ligands results in a better understanding of ligand-receptor interaction and, thereby, leads to new classes of H_3 ligands.

Chapter 3 describes the synthesis and H_3 activity of a conformationally constrained histamine derivative. This rigid compound enables molecular modelling studies that are described in chapter 4. A model is presented that reveals a molecular determinant for efficacy and, in addition, gives an explanation for the differences in structure-activity relationships observed for different classes of H_3 antagonists. Chapter 5 describes a new H_3 antagonist that has a remarkable activity profile. The synthesis and pharmacological activities of some derivatives of this antagonist are also described, to illustrate the predictive power of the aforementioned theoretical model. In chapter 6 the development of new partial agonists for the H_3 receptor is described and, thereby, the limitations of the model described in chapter 3 are indicated. Two new series of compounds are described in chapter 7. These ligands unravel various molecular features that are important for H_3 binding and, in addition, give essential information about

the 3D orientation of these features. Chapter 8 deals with the development of a new, more detailed pharmacophore model for H₃ antagonists. Again, the accuracy of this model is tested by the design, synthesis and pharmacological evaluation of a series of compounds.

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Chapter 3

Various approaches to the synthesis of 2-(1*H*-imidazol-4-yl)cyclopropylamine and histaminergic activity of (1*R*,2*R*)- and (1*S*,2*S*)-2-(1*H*-imidazol-4-yl)cyclopropylamine

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Abstract

*Various approaches towards the synthesis of all four stereoisomers of 2-(1*H*-imidazol-4-yl)-cyclopropylamine (cyclopropylhistamine) are described. The rapid and convenient synthesis and resolution of trans-cyclopropylhistamine is reported. The absolute configuration of its enantiomers was determined by single crystal X-ray crystallographic analysis. The distinct trans-cyclopropylhistamine enantiomers were tested for their activity and affinity on the histamine H_3 receptor. (1*S*,2*S*)-cyclopropylhistamine (VUF 5297) acts as an agonist both on the rat cortex ($pD_2=7.1$; $\alpha=0.75$) and on guinea-pig jejunum ($pD_2=6.6$; $\alpha=0.75$). Its enantiomer, (1*R*,2*R*)-cyclopropylhistamine (VUF 5296) is about one order of magnitude less active. Both enantiomers show weak activity on H_1 and H_2 receptors. All synthetic attempts to prepare cis-cyclopropylhistamine were unsuccessful. Nevertheless, the results of this study provide an ideal template for molecular modelling studies of histamine H_3 receptor ligands.*

Introduction

Next to its role as mediator in (patho)physiological processes, histamine acts a neurotransmitter.¹ The different pharmacological actions of histamine² are mediated *via* the activation of distinct histamine receptor subtypes. In addition to two postsynaptic receptor subtypes, H₁ and H₂, a presynaptic H₃ receptor has been identified. The histamine H₃ autoreceptor is responsible for controlling neuronal synthesis of histamine and in addition regulates the release of the neurotransmitter into the synaptical cleft.³ Furthermore, the receptor has been shown (as a heteroreceptor) to modulate the release of other neurotransmitters, *e.g.*, serotonin,^{4,5} acetylcholine,⁶ dopamine⁷ and noradrenaline,⁸ in both the central nervous system (CNS) and peripheral nervous system. Therefore the H₃ receptor can be considered a potential target for the development of new therapeutic agents, *e.g.*, to treat neurologic disorders.⁹ To determine the functional roles of the H₃ receptor, it is necessary to develop potent and selective H₃ ligands. Design of such compounds can be facilitated by molecular modelling. Furthermore, such studies can help to elucidate the molecular mechanisms involved in receptor stimulation. Therefore, the structural features that are responsible for affinity and intrinsic activity of a ligand need to be resolved.

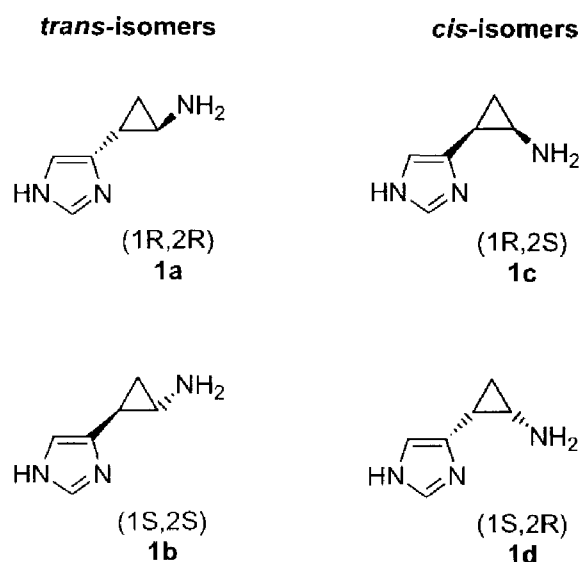


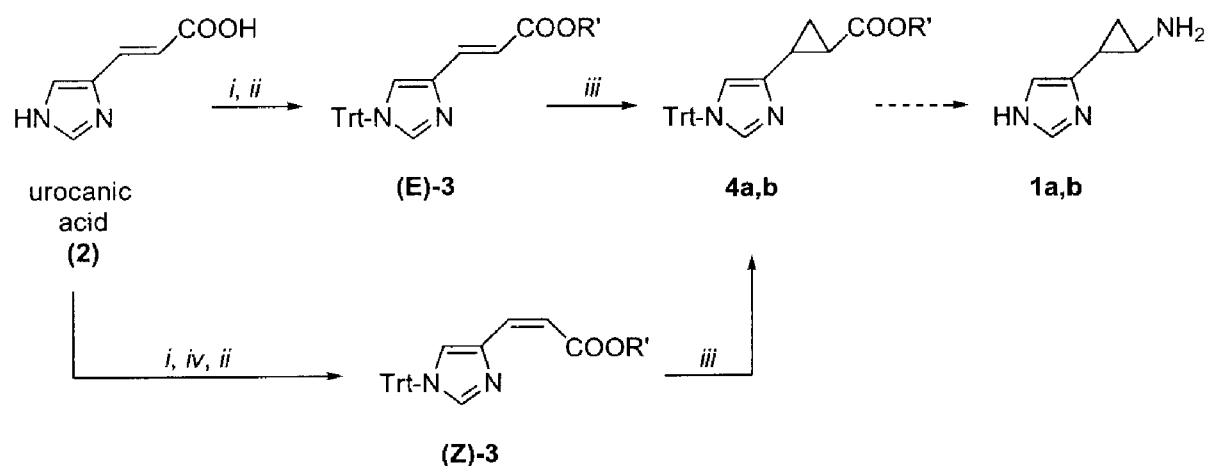
Figure 1. The stereoisomers of cyclopropylhistamine (**1**).

The development of rigid histamine analogues will contribute to the determination of the H₃ receptor pharmacophore, since the limited number of conformations of these compounds only allows a few spatial orientations of the pharmacophoric elements of H₃ receptor agonists, *i.e.*, the orientation of the imidazole ring with respect to the basic nitrogen in the side chain of the

ligands. A small and rigid compound such as 2-(1*H*-imidazol-4-yl)-cyclopropylamine (cyclopropylhistamine) (**1**) would be an ideal template for the development of a pharmacophore model, using molecular modelling techniques. Some preliminary pharmacological data for this compound have been reported in a patent application.¹⁰ Unfortunately, the stereochemical identity of the material tested was not reported. Hence, we speculated that the results reported by Arrang and co-workers could be based on experiments with racemic mixtures of both the *trans*- and the *cis*-diastereoisomers (denoted here as **1a,b** and **1c,d** respectively; Figure 1). As the H₃ receptor is highly stereoselective,^{11,12} it is reasonable to assume that each of the stereoisomers will have a different activity. Obviously, for molecular modelling studies, the potencies of each of the distinct isomers has to be determined. Therefore, we tried to synthesise the four stereoisomers of cyclopropylhistamine and to study their respective H₃ pharmacology.

Chemistry

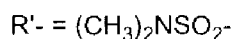
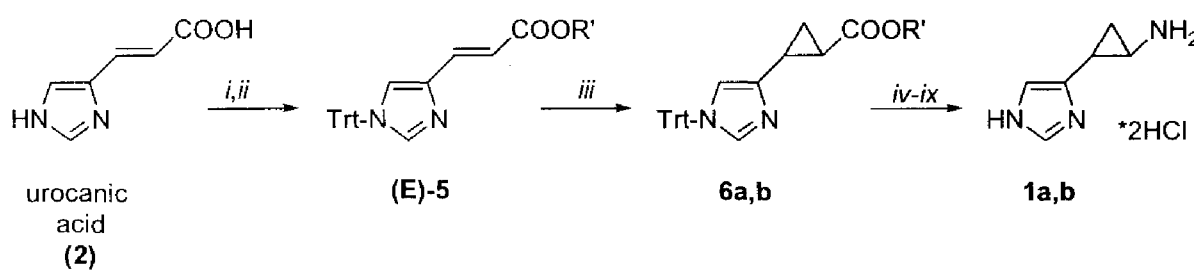
In the aforementioned patent application by Arrang *et al.*,¹⁰ cyclopropylhistamine (**1**) was obtained via a route developed by Burger and co-workers.¹³ In this route, cyclopropylhistamine (**1**) was synthesised starting from urocanic acid (**2**), via cyclopropanation of *sec*-butyl *trans*-3-(1-trityl-1*H*-imidazol-4-yl)acrylate (**E**)-(**3**) with dimethyloxosulfonium methylide followed by a Curtius rearrangement of the carboxylate group (Scheme 1). In our hands, the key-step in this synthesis scheme, the cyclopropanation, yielded a racemic mixture of *trans*-cyclopropane **4a,b** in a moderate yield (41%).



Scheme 1. (i) *sec*-butanol, H₂SO₄; (ii) Trt-Cl, Et₃N, CH₂Cl₂; (iii) Me₃SO⁺I⁺, NaH, DMF; (iv) λ=254 nm, CH₂Cl₂.

No cyclopropane product with the *cis*-configuration was detected in the reaction mixture. Efforts to obtain *cis*-cyclopropylhistamine (**1c,d**) via cyclopropanation of the isomeric *sec*-butyl *cis*-3-(1-trityl-1*H*-imidazol-4-yl)-acrylate¹⁴ (**Z**)-(3) as starting material also failed. Only *trans*-cyclopropanane product **4a,b** was obtained. Variation of the reaction conditions and the use of other protective groups for the imidazole moiety or the carboxylate group could neither improve the yield nor change the stereochemical outcome of the reaction. The addition of sulfonium ylide to the double bond proceeds *via* a non-concerted reaction mechanism and theoretically the formation of a mixture of all four cyclopropane stereoisomers is possible. However, we have to conclude that the addition to urocanic acid derivatives exclusively leads to the thermodynamically most stable product having the *trans*-cyclopropane configuration, regardless of the configuration of the unsaturated bond in the starting material.

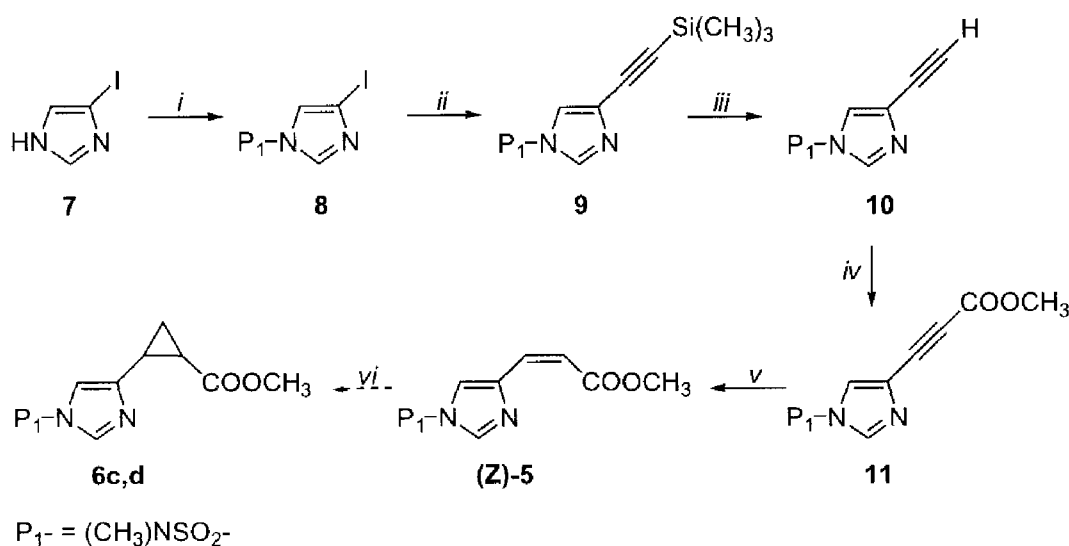
Subsequently, we investigated other methods to obtain this compound in a stereoselective manner. Cyclopropanation of the urocanate (**E**)-5 by the stereospecific addition of diazomethane, using palladium(II)acetate as a catalyst gave the *trans*-cyclopropane **6a,b** in a moderate yield (Scheme 2) as previously described by Vollinga. However, it must be noted that higher temperatures, larger amounts of catalyst and repeated application of the reaction conditions are required compared to the reported cyclopropanations of analogous non-imidazole α,β -unsaturated carboxylic esters.^{15,16,17} Alkaline hydrolysis of the ester **6a,b** and subsequent Curtius rearrangement yielded the racemic *trans*-cyclopropylhistamine (**1a,b**). Having obtained only a small amount of *trans*-cyclopropylhistamine (**1a,b**) we were unable to separate the enantiomers.



Scheme 2. Reagents used: (i) $\text{HCl}_{(\text{g})}$, MeOH, reflux; (ii) dimethylsulfamoylchloride, Et_3N , DCM; (iii) CH_2N_2 , cat. $\text{Pd}(\text{OAc})_2$, DCM; (iv) 1M KOH, MeOH, THF; (v) ClCOOEt , Et_3N , acetone; (vi) NaN_3 , H_2O ; (vii) toluene, reflux; (viii) *tert*-butanol, reflux; (ix) 1M HCl, reflux.

For the synthesis of the *cis*-isomers of cyclopropylhistamine (**1c,d**), the intermediate *cis*-urocanate (**Z**)-5 was prepared by a new synthetic pathway (scheme 3). 4-Iodo-1*H*-imidazole

(7) protected with an *N,N*-dimethylsulfamoyl group was coupled to trimethylsilylacetylene in a palladium-catalysed reaction.^{17,18} Removal of the trimethyl silyl group by treatment with KOH gave the acetylenic derivative **10** which was treated with ethylmagnesium bromide and dimethylcarbonate to provide alkyne ester **11**. Subsequent hydrogenation over Lindlar catalyst¹⁹ yielded the *cis*-urocanate (**Z**)-**5**. However, palladium-catalysed cyclopropanation of this compound failed. All attempts to perform this reaction under the same, or even more drastic conditions compared to the *trans*-urocanate (**E**)-**5** failed to give the desired product; only starting material was recovered.

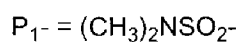
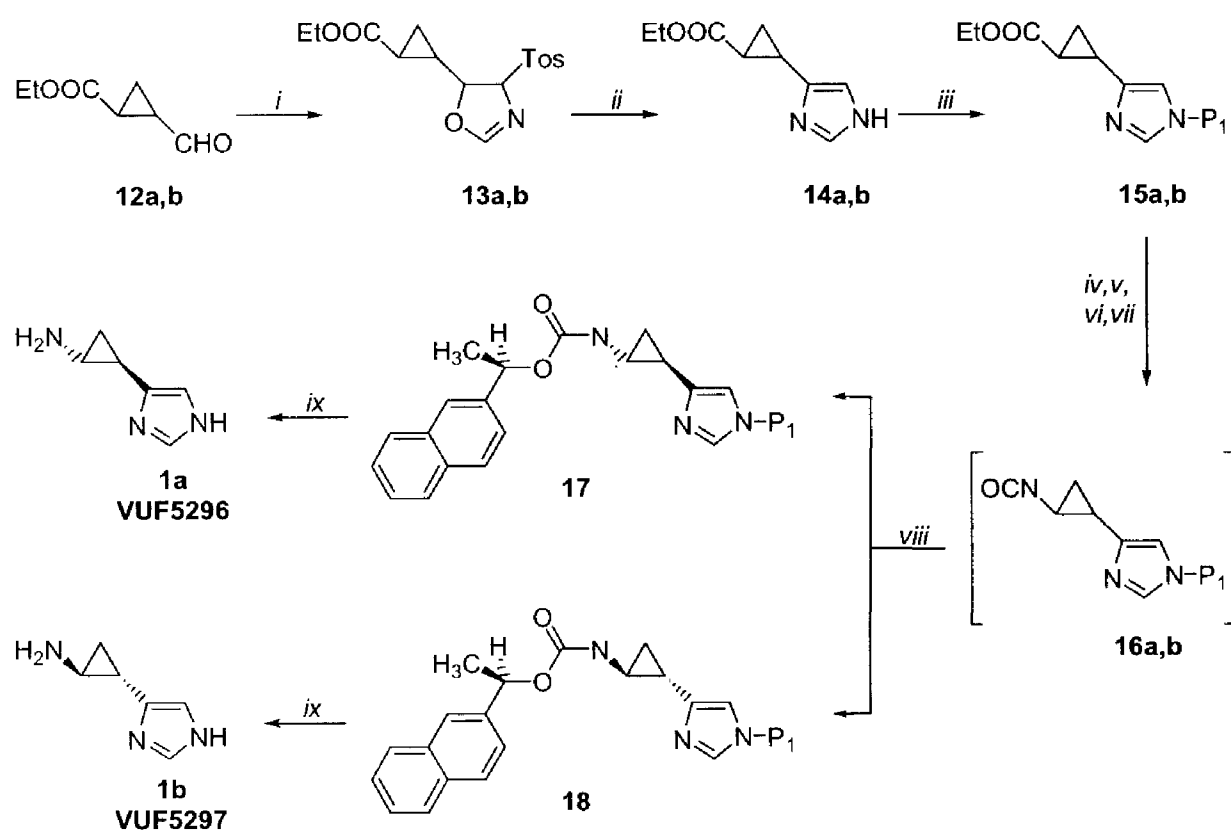


Scheme 3. Reagents used: (i) dimethylsulfamoylchloride, Et₃N, toluene; (ii) trimethylsilyl-acetylene, cat [(C₆H₅)₃PdCl₂, cat. CuI, Et₃N, 50°C; (iii) 2M KOH, MeOH, THF; (iv) EtMgBr, (CH₃O)₂CO; (v) H₂, Lindlar cat., quinoline, acetone; (vi) CH₂N₂, cat. Pd(OAc)₂, DCM

Other metal-catalysed cyclopropanation procedures (*e.g.*, Simmons-Smith reactions) on *trans*-urocanic acid derivatives such as (**E**)-**5** gave even lower yields of *trans*-cyclopropanated product. When using *cis*-urocanic acid derivatives such as (**Z**)-**5**, these methods failed completely to give cyclopropanated product. We suggest that the transition-metal-catalysed cyclopropanations are seriously hampered because of chelation of the catalyst by the imidazole moiety.

It had to be concluded that the strategy to synthesise all isomers of cyclopropylhistamine (**1**) by cyclopropanation of imidazole-containing precursors suffered from serious drawbacks. The *trans*-isomers (**1a,b**) were obtained in only a moderate yield. The limited amount of the racemic compound was too small to separate into the enantiomers. Furthermore, all attempts to synthesise the *cis*-isomers (**1c,d**) failed.

Therefore, we changed our strategy and developed an alternative synthesis route, constructing the imidazole ring on an appropriate cyclopropyl precursor (scheme 4). Commercially available racemic ethyl *trans*-2-formyl-1-cyclopropane-carboxylate (**12a,b**) was allowed to react with (p-tolylsulfonyl)methyl isocyanide (TosMIC) in a [3+2] anionic cycloaddition.²⁰ The labile 4-tosyloxazoline **13a,b** which precipitated from the reaction mixture was filtered and immediately treated with a saturated solution of ammonia in ethanol at 120 °C. Both reaction steps proceeded rapidly and in high yields.



Scheme 4. Reagents used: (i) Tosmic, NaCN, EtOH, 0 °C; (ii) EtOH/NH₃; 120 °C; 15 bar (iii) Me₂NSO₂-Cl, Et₃N, CH₂Cl₂; (iv) KOH, THF, MeOH; (v) ClCO₂C₂H₅, Et₃N, acetone; (vi) NaN₃, H₂O; (vii) toluene, Δ; (viii) R(+)-1-(2-naphthyl)ethanol, toluene; (ix) 30% HBr, Δ

The imidazole nucleus in **14a,b** was protected with a dimethylsulfamoyl group and the ester **15a,b** was hydrolysed under basic conditions. Subsequent Curtius rearrangement gave the isocyanate **16a,b** which was converted into the diastereomeric carbamates **17** and **18** using the

enantiopure (R)-(+)-1-(2-naphthyl)ethanol. The diastereomers were easily separated by flash column chromatography. The diastereomeric purity of the isolated stereoisomers was verified by HPLC analysis. The absolute configuration of **18** was determined by single-crystal X-ray analysis (Figure 2).

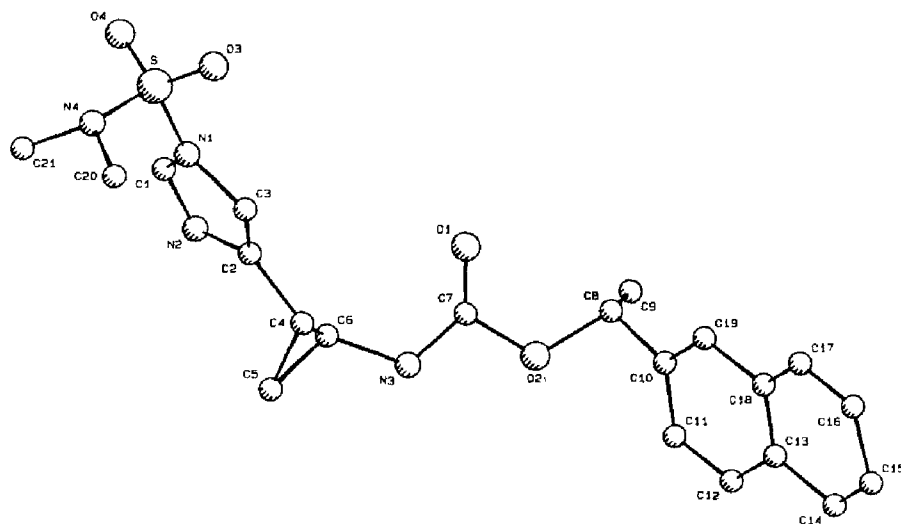


Figure 2. ORTEP drawing of **18**.

Hydrolysis of the distinct diastereoisomers **17** and **18** with hydrobromic acid gave the enantiopure *trans*-cyclopropylhistamines (**1a**) and (**1b**), respectively.

In an attempt to prepare *cis*-cyclopropylhistamine (**1c,d**) via an analogous route, racemic ethyl *cis*-2-formylcyclopropylcarboxylate²¹ reacted with TosMIC. This resulted in the precipitation of a highly unstable compound that could not be characterised. Immediate treatment of this material with ammonia in ethanol resulted in the formation of tars from which no cyclopropylimidazoles could be isolated or identified.

Pharmacological data.

The agonistic activities of the compounds **1a** and **1b** on the histamine H₃ receptor were determined on an *in vitro* test system based on the electrically-evoked contractile response of isolated guinea pig jejunum segments (Table 1).²² The receptor activities were also determined by measuring the release of [³H]-noradrenaline from electrically stimulated rat cerebral cortex slices.²³ Receptor affinities were determined using an H₃ receptor binding

assay on rat cortex (for details, see the Experimental Section). The compounds were additionally tested for H₂ agonism on the spontaneously-beating guinea pig right atrium²⁴ and for H₁ agonism on guinea pig ileum segments.²⁵

Table 1. Agonistic activities, intrinsic activity (α) and receptor affinity of the enantiopure *trans*-cyclopropylhistamines.

compound	configuration	H ₃			H ₂	H ₁
		pD ₂ ^a	pD ₂ ^b	pK _i ^c	pD ₂ ^d	pD ₂ ^e
histamine		7.4 ± 0.1 (α =1.0)	7.4 ± 0.1 (α =1.0)	8.2±0.1	6.1 ± 0.1 (α =1.0)	6.6 ± 0.1 (α =1.0)
1a VUF 5296	(1R,2R)	5.75±0.39 (α =0.64)	6.15±0.08 (α =0.32)	7.60±0.1	4.9 ± 0.1 (α =0.9)	4.5 ± 0.1 (α =0.9)
1b VUF 5297	(1S,2S)	7.08±0.15 (α =0.71)	6.64±0.06 (α =0.75)	8.76±0.1	4.8 ± 0.1 (α =1.0)	5.0 ± 0.1 (α =1.2)

^a Functional H₃ receptor assay on rat cortex.

^b Functional H₃ receptor assay on guinea pig jejunum.

^c H₃ receptor binding assay on rat cortex using [³H]-N ^{α} -methylhistamine.

^d Functional H₂ receptor assay on guinea pig atrium.

^e Functional H₃ receptor assay on guinea pig jejunum.

Discussion

The histamine H₃ receptor affinity of cyclopropylhistamine (**1**) has been reported in a patent application by Arrang *et al.*¹⁰ We assume that the material tested by Arrang and co-workers was a racemic mixture of *trans*-cyclopropylhistamine (**1a,b**). In our efforts to synthesise all four stereoisomers, we have developed a new route for the large scale synthesis and resolution of *trans*-cyclopropylhistamine (**1a,b**). However, all attempts to prepare *cis*-cyclopropylhistamine (**1c,d**) were unsuccessful.

Pharmacological evaluation of the enantiopure products (**1a**) and (**1b**) confirmed the stereoselectivity of H₃ receptors. We established (1S,2S)-2-(1*H*-imidazol-4-yl)-cyclopropylamine (**1b**) (VUF 5297) as the eutomer, having a moderate H₃ activity. Its enantiomer VUF 5296 is about 10 times less potent. Both stereoisomers show partial agonistic activity. Only minor differences in potencies between the two functional assays were found and no significant differences were found between the functional assays and the receptor

binding assay. Therefore, the suggested H₃ receptor heterogeneity² is not manifested by the compounds (**1a**) and (**1b**).

During the preparation of this manuscript, Khan *et al.*²⁶ reported the synthesis of *trans*-cyclopropylhistamine (**1a**) and (**1b**) via a diastereomeric synthesis route analogous to the route developed by Burger *et al.*¹³ Surprisingly, they reported (1*R*,2*R*)-2-(1*H*-imidazol-4-yl)-cyclopropylamine (**1a**) to be the most active enantiomer. Unfortunately, no analytical characterisation of the ligands was reported by Khan and co-workers; we were therefore unable to compare the optical rotation of the cyclopropylhistamines prepared in the different laboratories. However, a modelling study by Sippl *et al.*²⁷ suggested that (1*S*,2*S*)-cyclopropylhistamine (**1b**) provided the best sterical agreement with the derived H₃ receptor pharmacophore model. Our present study supports these theoretical findings.

The rigid cyclopropylhistamine derivatives show only a moderate to low H₁ and H₂ receptor activity. The histamine H₁ receptor has a slight preference for the (1*S*,2*S*)-enantiomer **1b**.

Conclusion

A rapid and convenient synthesis and resolution of *trans*-2-(1*H*-imidazol-4-yl)-cyclopropylamine (*trans*-cyclopropylhistamine) is described. (1*S*,2*S*)-2-(1*H*-imidazol-4-yl)-cyclopropylamine (VUF 5297) (**1b**) is a rigid H₃ receptor agonist about ten times more active than its enantiomer. These results have enabled us to construct an unambiguous pharmacophore model for the histamine H₃ receptor, explaining agonistic and antagonistic activity of histamine H₃ ligands, as will be described in chapter 4.²⁸

All attempts to synthesise *cis*-cyclopropylhistamine were unsuccessful.

Experimental Section

Chemistry.

¹H- and ¹³C-NMR spectra were recorded on a Bruker AC-200 spectrometer with tetramethylsilane as an internal standard. Mass spectra were recorded on a Finnigan MAT-90. Melting points were determined on a Mettler FP-5 + FP-52 apparatus and are uncorrected. GLC-Analysis was performed on a Shimadzu GC-14A equipped with an FID detector and an HP1 column (50 m x 0.31 mm). Elemental analyses were performed by Micro Kemi AB, Uppsala, Sweden. Solvents were purified and dried by standard procedures before use.

Methyl (E)-3-(1*H*-imidazol-4-yl)-acrylate

Urocanic acid (25.0 g, 0.18 mol) was added to refluxing methanol (150 ml) and HCl-gas was bubbled through the mixture, according to literature procedures.²⁹ After refluxing for 5h, the clear solution was cooled down to 5 °C and white needle-like crystals appeared (32.20 g, 94%). Melting point 233.5-234.5 °C.

Methyl (E)-3-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-acrylate (5)

Methyl (E)-3-(1*H*-imidazol-4-yl)-acrylate (32.20 g, 0.17 mol) was dissolved in a solution of dichloromethane (600 ml) and triethylamine (75 ml). After the addition of *N,N*-dimethylsulfamoyl-chloride (20.0 ml, 0.19 mol), the reaction mixture was refluxed for 48 h. The solution was washed with H₂O (150 ml) and saturated aqueous sodium chloride (150 ml). After evaporation of the dichloromethane, a white solid (43.37 g) remained. Recrystallisation from *iso*-propanol resulted in white crystals (37.55 g, 85%). Melting point 137.0-138.0 °C.

¹H NMR (CDCl₃): δ 2.89 (s, 6H, NCH₃), 3.80 (s, 3H, OCH₃), 6.67 (d, *J*=15.6 Hz, 1H), 7.38 (s, 1H), 7.52 (d, *J*=15.6 Hz, 1H), 7.90 (s, 1H) ppm.

¹³C NMR (CDCl₃): δ 38.2, 51.7, 118.7, 119.1, 134.4, 137.5, 139.3, 167.3 ppm.

Anal. (C₉H₁₃N₃O₄S) C, H, N.

***Trans*-2-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-cyclopropanecarboxylic acid**

A solution of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazogen) (40.0 g, 0.19 mol) dissolved in ether (200 ml) was added dropwise to a heated mixture of KOH (31.0 g, 0.55 mol in 200 ml H₂O) and 2(2-ethoxyethoxy)ethanol (200 ml); special “soft” glassware was used. The hence generated etheric diazomethane solution was distilled dropwise into a solution of (**E**)-**5** (5.0 g, 19.3 mmol) and Pd(OAc)₂ (200 mg) in dichloromethane (1 L) at room temperature. The light yellow solution became dark brown and N₂-evolution was observed. The reaction mixture was stirred overnight. After the addition of a few drops acetic acid, the reaction mixture was washed with 5% NaHCO₃-solution (250 ml). The organic layer was dried over Na₂SO₄, filtered over a short silica column and concentrated *in vacuo*. The remaining light yellow oil consisted of 49% product **6a,b** and 51 % starting material (GC and ¹H NMR). The residue was dissolved in dichloromethane (1 L) and the procedure was repeated, employing the same amounts of reagents. This time the residual oil consisted of 82% cyclopropane product and 18% starting material.

¹H NMR (CDCl₃) **6a,b**: δ 1.42-1.57 (m, 2H), 2.03-2.13 (m, 1H), 2.30-2.48 (m, 1H), 2.84 (s, 6H), 3.70 (s, 3H), 7.08 (s, 1H), 7.77 (s, 1H) ppm.

The crude product was dissolved in a mixture of methanol (100 ml) and tetrahydrofuran (100 ml). A portion of 1M KOH-solution (100 ml) was added and after 0.5 h stirring at room temperature, the mixture was washed with dichloromethane (3x200 ml). The aqueous layer was treated with 1M HCl-solution until the mixture had a pH of 2. The product was extracted with dichloromethane (3x200 ml) and after drying over Na₂SO₄, filtration and evaporation of the dichloromethane, a light brown solid (3.91 g) remained. After several triturations with dry acetone, a white powder (2.92 g, 58 %) was collected. Melting point 166.0 °C.

¹H NMR (DMSO-d₆) : δ 1.28-1.37 (m, 2H), 1.78-1.88 (m, 1H), 2.28-2.38 (m, 1H), 2.78 (s, 6H), 7.50 (s, 1H), 8.00 (s, 1H), 12.28 (bs, 1H) ppm. ¹³C NMR (DMSO-d₆) : δ 14.8, 18.7, 22.0, 37.7, 114.1, 136.7, 141.5, 173.8 ppm. *Anal.* (C₉H₁₃N₃O₄S) C, H, N.

***trans*-2-(1*H*-Imidazol-4-yl)-cyclopropylamine (1a,b)**

Racemic *trans*-2-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-cyclopropanecarboxylic acid (2.22 g, 8.6 mmol) was dissolved in dry acetone (65 ml) and triethylamine (1.6 ml, 11.5 mmol) under N₂ atmosphere. The reaction mixture was cooled to 0 °C and ethyl chloroformate (1.6 ml, 16.7 mmol) was added dropwise (a white precipitate formed). After 2h, a solution of sodium azide (0.85 g, 13.1 mmol in 15 ml H₂O) was added slowly and the mixture was stirred for another hour. After addition of H₂O (65 ml), the solution was concentrated *in vacuo* until the water layer remained. The water layer was extracted with toluene (3x65 ml) and the combined toluene layers were dried over Na₂SO₄, filtered and refluxed for 2h (N₂-evolution was observed). The residue after evaporation of toluene was refluxed in *tert*-butanol (50 ml) for 12 h (disappearance of the isocyanate-peak in IR spectrum). After evaporation of the alcohol a dark residue remained. This residue was dissolved in ethyl acetate and filtered over a short silica column. A white solid (2.10 g) remained after evaporation of the ethyl acetate. This was refluxed for 12 h in 1M HCl (100 ml). After concentration *in vacuo*, the residue was refluxed in absolute ethanol (60 ml) for 0.5 h, concentrated and subsequently washed with acetone. A beige solid (1.06 g, 63%) remained, which was recrystallised from iso-propanol/ether. Melting point 187.0-187.5 °C.

¹H NMR (D₂O): δ 1.42 (ddd, *J*=6.7, 6.9 and 8.2 Hz, 1H), 1.55 (ddd, *J*=4.7, 7.2 and 10.2 Hz, 1H), 2.51 (ddd, *J*=3.4, 6.5 and 10.1 Hz, 1H), 3.03 (ddd, *J*=3.6, 4.5 and 8.2 Hz, 1H), 7.25 (s, 1H), 8.57 (s, 1H) ppm. ¹³C NMR (D₂O): δ 12.4, 12.5, 30.7, 117.4, 132.4, 134.9 ppm. *Anal.* (C₆H₉N₃·2HCl) C, H, N.

1-(*N,N*-Dimethylsulfamoyl)-4-iodo-1*H*-imidazole (8)

N,N-Dimethylsulfamoylchloride (12.0 ml, 0.11 mol) was added to a solution of 4-iodo-1*H*-imidazole (7) (20.0 g, 0.10 mol) in dichloromethane (600 ml) and triethylamine (40 ml). The

reaction mixture was refluxed for 60 h and subsequently washed with H₂O (250 ml) and 5% Na₂S₂O₃-solution (250 ml). A light brown solid (30.22 g) remained after evaporation of the solvents. Recrystallisation from hot *iso*-propanol resulted in white crystals (8.65 g, 60%). Melting point 131.5 °C.

¹H NMR (CDCl₃): δ 2.89 (s, 6H), 7.34 (s, 1H), 7.78 (s, 1H) ppm. ¹³C NMR (CDCl₃): δ 38.2, 84.4, 122.7, 137.8 ppm. *Anal.* (C₅H₈N₃O₂Si) C, H, N.

Methyl (1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-propynoate (11)

To a solution of **8** (20.0 g, 67 mmol) in triethylamine (250 ml) was added bis(triphenylphosphine)paladium(II)chloride (1 mol%, 0.5 g), CuI (1 mol%, 130 mg) and trimethylsilylacetylene (20.0 ml, 141 mmol). This reaction mixture was stirred for 60 h on a 50 °C oil bath. After filtration of the reaction mixture, the solution was concentrated *in vacuo*.

¹H NMR (CDCl₃) **9**: δ 0.25 (s, 9H), 2.87 (s, 6H), 7.40 (s, 1H), 7.81 (s, 1H) ppm.

The residue **9** was dissolved in methanol (150 ml) and tetrahydrofuran (150 ml). A solution of 2M potassium hydroxide (150 ml) was added and the mixture was poured in a saturated NH₄Cl-solution (150 ml). After extraction of the product with dichloromethane (1x300 ml; 2x100ml), drying over Na₂SO₄, filtration and evaporation of the solvent, a brown oil (12.3 g) remained. The oil was purified by column chromatography using ethyl acetate as eluent (R_f=0.7). A light beige solid (1-(*N,N*-dimethylsulfamoyl)-4-ethynyl-1*H*-imidazole) (7.82 g, 59%) was isolated. ¹H NMR (CDCl₃) **10**: δ 2.88 (s, 6H), 3.13 (s, 3H), 7.42 (s, 1H), 7.83 (s, 1H) ppm.

This beige solid **10** (5.0 g, 25 mmol) was dissolved in dichloromethane (50 ml) and added dropwise to a Grignard mixture, prepared from magnesium (0.8 g, 33 mmol) and ethyl bromide (2.5 ml, 33 mmol) in tetrahydrofuran (5 ml). After 1h the mixture was poured in dimethylcarbonate (100 ml) and stirred overnight. The solution was poured in H₂O (250 ml) and extracted with dichloromethane (3x200 ml). After evaporation of the solvents, a brown oil (5.53 g) remained, which was purified on a silicagel column with ethyl acetate as eluent (R_f=0.8). After two recrystallisations from *iso*-propanol, white crystals (2.98 g, 46%) were collected. Melting point 126.0-127.0 °C.

¹H NMR (CDCl₃) **11**: δ 2.91 (s, 6H, NCH₃), 3.84 (s, 3H), 7.63 (s, 1H), 7.88 (s, 1H) ppm. ¹³C NMR (CDCl₃) **11**: δ 38.2, 52.9, 78.6, 82.1, 123.2, 124.6, 137.1, 154.0 ppm.

Anal. (C₉H₁₁N₃O₄S) C, H, N.

Methyl (Z)-3-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-acrylate (5)

Compound **11** (2.6 g, 10.1 mmol) was dissolved in acetone (75 ml). Lindlar catalyst (120 mg) and quinoline (300 mg) were added and the reaction mixture was stirred under hydrogen atmosphere (1 atm). After hydrogen consumption had ceased, additional Lindlar catalyst was added (repeated twice; a total of 280 mg catalyst extra was added). After 8 h and 300 ml of hydrogen gas consumption, the reaction mixture was filtered and concentrated *in vacuo*. The residue consisted of 92% *cis*- and 8% of the *trans*-isomer, according to GC. This was purified on a silicagel column with ethyl acetate as eluent ($R_f=0.5$) and recrystallisation from *n*-hexane. White crystals (1.8 g, 69%) were isolated. Melting point 74.0 °C.

^1H NMR (CDCl_3): δ 2.92 (s, 6H), 3.76 (s, 3H), 5.95 (d, $J=12.7$ Hz, 1H), 6.96 (d, $J=12.2$ Hz, 1H), 7.88 (s, 1H), 8.64 (s, 1H) ppm. *Anal.* ($\text{C}_9\text{H}_{13}\text{N}_3\text{O}_4\text{S}$) C, H, N.

Ethyl *trans*-2-(4-(methylphenyl)sulfonyl-4,5-dihydro-oxazol-5-yl)-cyclopropanecarboxylate (13a,b)

To a stirred suspension of tosylmethyl isocyanide (6.8 g, 34.6 mmol) and ethyl *trans*-2-formyl-1-cyclopropane-carboxylate (**12a,b**) (5.0 g, 35.2 mmol) in absolute ethanol (250 ml) at 0 °C was added sodium cyanide (45 mg). For a moment the reaction mixture became clear followed by precipitation of the product. Ten minutes after the addition of sodium cyanide, the suspension was filtered and product was washed with ether/hexane (20 ml, 1/1, v/v) and dried *in vacuo*. The product was isolated as a white hygroscopic solid (9.9 g, 85%). Melting point: 107.0 °C. ^1H NMR (CDCl_3): δ 0.96-1.06 (m, 1H), 1.25 (t, $J=7.14$ Hz, 3H), 1.55-1.77 (m, 2H), 1.79-1.89 (m, 1H), 2.55 (s, 3H), 4.12 (q, $J=7.14$ Hz, 2H), 4.72 (dd, 1H, $J=4.11, 4.11$ Hz), 4.92 (dd, $J=4.11, 1.63$ Hz, 1H), 6.97 (d, $J=1.63$ Hz, 1H), 7.36 (d, $J=8.16$ Hz, 2H), 7.80 (d, $J=8.16$ Hz, 2H) ppm. ^{13}C NMR (CDCl_3): δ 12.39, 14.00, 16.77, 21.55, 24.20, 60.84, 79.32, 89.72, 129.07, 129.72, 132.69, 145.56, 159.16, 172.37 ppm.

Ethyl *trans*-2-(1*H*-imidazol-4-yl)-cyclopropanecarboxylate (14a,b)

In a stainless steel bomb, a solution of oxazoline **13a,b** (9.0 g, 26.7 mmol) and saturated solution of ammonia in absolute ethanol (120 ml) was heated at 120 °C for 25 h. The pressure increased to 12 atm. After cooling, the solvent was removed under reduced pressure. The dark, oily residue was dissolved in ethyl acetate/dichloromethane (150 ml, 4/1, v/v) and washed with saturated aqueous sodium chloride (5x 50 ml). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo* to give the product as an oil (3.56 g, 74%).

^1H NMR (CDCl_3): δ 1.18 (t, $J=7.14$ Hz, 3H), 1.28-1.38 (m, 1H), 1.39-1.48 (m, 1H), 1.82-1.93 (m, 1H), 2.37-2.48 (m, 1H), 4.06 (q, $J=7.14$ Hz, 2H), 6.77 (s, 1H), 7.45 (s, 1H).

Ethyl *trans*-2-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-cyclopropanecarboxylate (15a,b)

To a solution of **14a,b** (3.00 g, 16.9 mmol) and triethylamine (5.0 ml, 36.1 mmol) in dichloromethane (70 ml) was added *N,N*-dimethylsulfamoylchloride (3.1 ml, 28.9 mmol). Subsequently, the reaction mixture was refluxed for 22 h. Concentration under reduced pressure followed by flash column chromatography (ethyl acetate/hexane, 5/3, v/v, $R_f=0.5$) gave the product as a yellow oil (4.13 g, 85%).

^1H NMR (CDCl_3): δ 1.26 (t, $J=6.67$ Hz, 3H), 1.37-1.58 (m, 2H), 1.98-2.10 (m, 1H), 2.36-2.48 (m, 1H), 2.87 (s, 6H), 4.14 (q, $J=6.67$ Hz, 2H), 7.04 (s, 1H), 7.72 (s, 1H) ppm.

***trans*-2-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-cyclopropanecarboxylic acid**

Compound **15a,b** (2.5 g, 8.69 mmol) was dissolved in a mixture of methanol (100 ml) and THF (100 ml). At room temperature, 1M KOH-solution (100 ml) was added and after 0.5 h stirring, the mixture was washed with dichloromethane (3x100ml). The water-layer was acidified with 1M HCl until a pH of 2 and extracted with dichloromethane (3x100ml). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo* to give a white powder (2.25 g, 75 %). Melting point 166.0 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 1.28-1.37 (m, 2H), 1.78-1.88 (m, 1H), 2.28-2.38 (m, 1H), 2.78 (s, 6H), 7.50 (s, 1H), 8.00 (s, 1H), 12.28 (bs, 1H) ppm. Anal. ($\text{C}_9\text{H}_{13}\text{N}_3\text{O}_4\text{S}$) C, H, N.

1'(*R*)-1-(2-Naphtyl)-ethyl *trans*-[2-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-cyclopropyl]-carbamate (17, 18)

To a solution of racemic *trans*-2-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-cyclopropanecarboxylic acid (4.44 g, 17.2 mmol) and triethylamine (3.2 ml, 3.0 mmol) in acetone (125 ml) at 0 °C was added dropwise ethyl chloroformate (3.2 ml, 33.4 mmol). After 2 h, a solution of sodium azide (1.70 g, 26.2 mmol) in H_2O (25 ml) was added slowly to the formed suspension and stirred for an additional hour. After addition of water (100 ml), the solution was extracted with toluene (3x100 ml). The organic layer was dried over sodium sulfate. After filtration, the solution was refluxed for 2h (N_2 -evolution was observed). Subsequently, *R*(+)-1-(2-naphtyl)ethanol (3.26 g, 18.9 mmol) was added. The mixture was refluxed for 13 h. After evaporation of the solvent, the diastereomeric carbamates were isolated using a short silica column (ethyl acetate, $R_f=0.6$) to give a white powder (6.41 g, 87 %). The ratio of diastereoisomers was determined using HPLC analysis as 50:50 (chrompack CPTm SpherSi, eluent ethyl acetate/hexane, 60/40, v/v at 1 ml/min; $p=20$ bar) (**11** has a retention time of 19.68 min and **12** has a retention time of 23.58 min).

Subsequent separation by flash column chromatography (ethyl acetate/hexane, 4/1, v/v, $R_f=0.26$) gave the separate diastereoisomers. The diastereomeric purity of the isolated compounds was verified by HPLC.

1'(R) 1-(2-Naphtyl)-ethyl (1R, 2R)-[2-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-cyclopropyl]-carbamate (17)

$R_f=0.60$ (ethyl acetate)

Melting point: 156.0-157.0 °C, $[\alpha]_{546}^{30} = +65.0^\circ$ (c 0.24, CHCl₃)

¹H NMR (CDCl₃) : δ 1.04-1.19 (m, 1H), 1.23-1.33 (m, 1H), 1.56 (d, $J=6.58$ Hz, 3H), 1.91-2.01 (m, 1H), 2.70 (s, 6H), 2.82-2.98 (m, 1H), 5.53 (bs, 1H), 5.94 (q, $J=6.58$ Hz, 1H), 6.97 (s, 1H), 7.44-7.46 (m, 3H), 7.72-7.80 (m, 5H) ppm. ¹³C NMR (CDCl₃): δ 14.68, 18.10, 22.27, 31.74, 37.89, 72.77, 112.99, 123.92, 124.66, 125.83, 126.03, 127.48, 127.85, 128.13, 132.78, 132.99, 136.05, 139.28, 142.89, 156.31 ppm.

(1'R) 1-(2-Naphtyl)-ethyl (1S, 2S)-[2-(1-(*N,N*-dimethylsulfamoyl)-1*H*-imidazol-4-yl)-cyclopropyl]-carbamate (18)

$R_f=0.57$ (ethyl acetate)

Melting point: 157.0-158.5 °C, $[\alpha]_{546}^{30} = +5.6^\circ$ (c 0.24, CHCl₃).

¹H NMR (CDCl₃) : δ 1.04-1.19 (m, 1H), 1.23-1.33 (m, 1H), 1.58 (d, $J=6.58$ Hz, 3H), 1.91-2.01 (m, 1H), 2.75 (s, 6H), 2.82-2.98 (m, 1H), 5.30 (bs, 1H), 5.95 (q, $J=6.58$ Hz, 1H), 6.99 (s, 1H), 7.44-7.46 (m, 3H), 7.72-7.80 (m, 5H) ppm. ¹³C NMR (CDCl₃): δ 14.66, 18.10, 22.40, 31.19, 37.95, 72.88, 76.31, 76.94, 77.58, 112.98, 123.89, 124.65, 125.84, 126.03, 127.48, 127.86, 128.12, 132.78, 133.00, 136.05, 139.22, 142.88, 156.24 ppm.

Single crystals of this diastereoisomer **18** were obtained by recrystallisation from ethanol. It is noted that (pure) diastereoisomer **17** did not crystallise in this solvent.

X-ray crystal data for 18:

C₂₁H₂₄N₄O₄S, $M_r=428.5$, monoclinic, P2₁, $a=13.930$ (1) Å, $b=5.4967$ (4) Å, $c=15.928$ (1) Å, $V=1138.7$ (1) Å³, $Z=2$, $D_x=1.25$ gcm⁻³, $\lambda(\text{CuK}\alpha)=1.5418$ Å, $\lambda(\text{CuK}\alpha)=15.01$ cm⁻¹, $F(000)=452$, room temperature. Final $R=0.096$ for 876 observed reflections.

A crystal with dimensions 0.03 x 0.05 x 0.60 mm approximately was used for data collection on an Enraf-Nonius CAD-4 diffractometer with graphite-monochromated CuK α radiation and ω -2 θ scan. A total of 2403 unique reflections were measured within the range $-16 \leq h \leq 15$, $0 \leq k < 6$, $0 \leq l \leq 19$. Of these, 876 were above the significance level of $2.5 \sigma(I)$. The range of $(\sin \theta)/\lambda$ was 0.034 - 0.609 Å⁻¹ ($3.0 < \theta < 69.9^\circ$). Two reference reflections (203, 212) were measured hourly and showed no decrease during the 57 h collecting time. In addition, 611

“Friedel” reflections were measured, which were used in the determination of the absolute configuration. Unit-cell parameters were refined by least-squares fitting procedure using 23 reflections with $68 < 2\theta < 78^\circ$. Corrections for Lorentz and polarisation effects were applied. The structure was solved by the PATTY option of the DIRDIF94 program system.³⁰ The hydrogen atoms restraining the latter in such way that the distance to their carrier remained constant at approximately 1.0 Å and keeping their displacement factors fixed at $U = 0.1$ Å, converged to $R=0.096$, $R_w=0.123$, $(\Delta/\sigma)_{\max}=0.09$, $S=1.07$. A weighting scheme $w=[10. + 0.01*(\sigma(F_{\text{obs}}))^2 + 0.0001/(\sigma(F_{\text{obs}}))]^{-1}$ was used. The secondary isotropic extinction coefficient^{31,32} refined to $\text{Ext}=0.08$ (3). The absolute structure parameter³³ refined to $X_{\text{abs}}=-0.04$ (20) thus confirming the correct enantiomer. A final difference Fourier map revealed a residual electron density between -0.7 and 1.3 eÅ⁻³ in the vicinity of the S-atom. Scattering factors were taken from Cromer and Mann³⁴ International Tables for X-ray Crystallography (1974). The anomalous scattering of S was taken into account. All calculations were performed with XTAL3.4,³⁵ unless stated otherwise.

(1R, 2R)-2-(1H-Imidazol-4-yl)-cyclopropylamine (VUF 5296) (1a)

A solution of **17** (1.25 g, 2.92 mmol) in 30% HBr (75 ml) was refluxed for 22 h. After concentration *in vacuo*, the residue was refluxed in absolute ethanol for 0.5 h and subsequently washed with acetone. White crystals (0.64 g, 77%) were collected.

Melting point: 207.0-208.0° C, $[\alpha]_{546}^{30} = +54.4^\circ$ (c 0.35, H₂O).

¹H NMR (D₂O): δ 1.42 (ddd, $J=6.7, 6.9$ and 8.2 Hz, 1H), 1.55 (ddd, $J=4.7, 7.2$ and 10.2 Hz, 1H), 2.51 (ddd, $J=3.4, 6.5$ and 10.1 Hz, 1H), 3.03 (ddd, $J=3.6, 4.5$ and 8.2 Hz, 1H), 7.25 (s, 1H), 8.57 (s, 1H) ppm. *Anal.* (C₆H₁₁N₃Br₂) C, H, N.

(1S, 2S)-2-(1H-Imidazol-4-yl)-cyclopropylamine (VUF 5296) (1b)

A solution of **18** (1.00 g, 2.33 mmol) in 30% HBr (75 ml) was refluxed for 22 h. After concentration *in vacuo*, the residue was refluxed in absolute ethanol for 0.5 h and subsequently washed with acetone. White crystals (0.51 g, 76%) were collected.

Melting point: 207.0-208.0°, $[\alpha]_{546}^{30} = -54.2^\circ$ (c 0.35, H₂O).

¹H NMR (D₂O): δ 1.42 (ddd, $J=6.7, 6.9$ and 8.2 Hz, 1H), 1.55 (ddd, $J=4.7, 7.2$ and 10.2 Hz, 1H), 2.51 (ddd, $J=3.4, 6.5$ and 10.1 Hz, 1H), 3.03 (ddd, $J=3.6, 4.5$ and 8.2 Hz, 1H), 7.25 (s, 1H), 8.57 (s, 1H) ppm. *Anal.* (C₆H₁₁N₃Br₂) C, H, N.

Pharmacology.

The compounds were tested for their activity on the histamine receptors in different assays (*vide supra*). These assays have been described in detail.²²⁻²⁵

The histamine H₃ receptor affinity was determined on rat cortical membranes with [³H]-N^α methylhistamine (81.9 Ci/mmol, NEN life science products, Brussels, Belgium) according to the method of West *et al.*³⁶ with modifications. Briefly, animals were killed by decapitation, and the cerebral cortex rapidly removed. Rat cortices were homogenised in 15 volumes (wt/vol.) of ice-cold Tris/HCl buffer (50mM Tris/HCl; 5mM MgCl₂, 145mM NaCl; pH 7.4 at 4°C) using an Ultra-Turrax homogeniser (8 seconds) and a glass-Teflon homogeniser (four strokes up and down) subsequently. All subsequent steps were carried out at 0°C to 4°C. The homogenate was centrifuged at 800 g for 10 minutes. The pellets were discarded and the supernatant was centrifuged for 20 minutes at 40,000 g. The resulting pellet was resuspended and the last centrifugation step was repeated. The pellet was resuspended in 1.5 volume (wt/vol.) Tris/HCl buffer to give a final concentration of ~ 300 µg/100 µl and stored in aliquots at -80°C. Protein concentration was determined using Biorad protein assay (Bio-Rad laboratories GmbH, Munich, Germany). Competition binding experiments were carried out in polypropylene tubes in a total volume of 400 µl of 50 mM Na⁺ phosphate buffer pH 7.4 at 37°C, containing 30 µg of protein, 1nM of [³H]-N^α methylhistamine and 0.1 to 10,000 nM of the compound to be tested. Samples were incubated for 40 minutes at 25°C. The incubation was started by the addition of 100 µl membranes (30 µg) and terminated by rapid filtration through polyethyleneimine (0.3% wt/vol.) pretreated Whatman GF/C filters using a Brandel filtration apparatus. The filters were washed twice with 3 ml of ice-cold Tris/HCl buffer (50 mM Tris/HCl; 5mM MgCl₂, 145mM NaCl; pH 7.4 at 4°C). The radioactivity retained on the filters was measured using liquid scintillation counting. Competition isotherms were analysed with the GraphPad Prism software (GraphPad™, Intuitive Software for Science, San Diego, CA, USA) K_i values were determined with the equation $K_i = IC_{50}/(1+([ligand]/K_d))$.

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Chapter 4

A qualitative model for the histamine H₃ receptor explaining agonistic and antagonistic activity of H₃ ligands

Abstract

A pharmacophore model for histamine H₃ ligands is derived that reveals the putative interaction of both H₃ agonists and antagonists with an aspartate residue of the receptor. This interaction is determined by applying the density functional theory implemented in a program package adapted for parallel computers. The model reveals a molecular determinant explaining efficacy as the conformation of the aspartic acid residue differs according to whether it is binding to agonists or antagonists. The differences in structure-activity relationships (SAR) for the lipophilic tails of the different classes of H₃ antagonists can now be explained, since the model reveals two distinct lipophilic pockets available for antagonist binding.

Introduction

Histamine is not only a mediator of several (patho)physiological processes, but also acts as a neurotransmitter.¹ Feedback mechanisms are crucial to neurotransmission and the presynaptic histamine H₃ receptor not only plays a key role in regulating histamine release but also regulates the release of other neurotransmitters such as acetylcholine,² dopamine,³ serotonin^{4,5} and noradrenaline.⁶ The highest density of H₃ receptors is found in the central nervous system, implying that the main pharmacological targets are found in the brain.⁷ However, a thorough investigation of the possible use of H₃ ligands as therapeutic agents is hampered by their poor blood-brain barrier penetration⁸ and their interaction with cytochrome P450 isoenzymes.⁹ Therefore, new ligands with improved pharmacokinetic profiles are needed.

Molecular modelling studies facilitate drug design and lead optimisation. Furthermore, these studies may contribute to the unravelling of the molecular mechanisms involved in receptor stimulation. Information about the H₃ receptor protein is limited. Because inhibitory effects on histamine H₃ receptor-mediated stimuli by G protein toxins (both cholera and pertussis toxin) have been reported, it is most likely that the histamine H₃ receptor belongs to the superfamily of G protein-coupled receptors (GPCRs)^{10,11}, *i.e.*, coupled to a G protein of the G_i

class.¹² In general, the hydrophobicity profile¹³ of the primary amino-acid sequence of G-protein coupled receptors indicates that these receptors contain seven hydrophobic transmembrane α -helices (Figure 1).

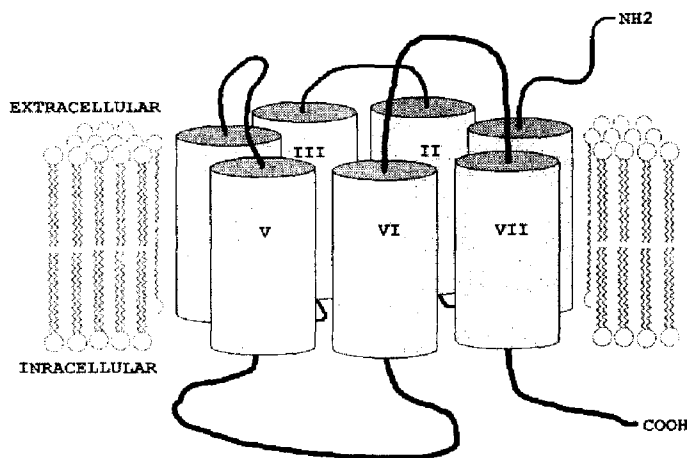


Figure 1. Schematic representation of the 3-D structure of a G-protein coupled receptor.

This seven α -helical motif is also revealed in the experimentally determined three-dimensional structure of bovine rhodopsin, a mammalian GPCR.^{13,14} The transmembrane-domains (TM) are connected by hydrophilic intra- and extracellular loops. In general, ligand binding to GPCRs is contained within the cavity formed by the seven transmembrane helices (Figure 2).

Several studies have indicated that the H_3 receptor can be classified as a member of the biogenic amine receptor subfamily of G-protein-coupled receptors (GPCRs).^{11,12,15} Receptors of this subfamily have a highly conserved aspartic acid residue (Asp) in transmembrane domain (TM) III,¹⁶ that is involved in binding the positively charged amino group of the endogenous ligands and is therefore seen as the main anchoring point for agonist binding.¹⁷ The recent cloning of the human histamine H_3 receptor by Lovenberg and co-workers verified the presence of the conserved Asp in TM III.¹⁸ Residues in other TM domains (especially in TM V) appear to be involved in determining the selectivity of these aminergic receptors for their endogenous agonists, *e.g.*, in the β_2 adrenergic receptor two serines in TM V are thought

to interact with the agonist catechol moiety¹⁹ and in the histamine H₁ receptor Lys and Asn residues in TM V are proposed to interact with the imidazole ring.²⁰

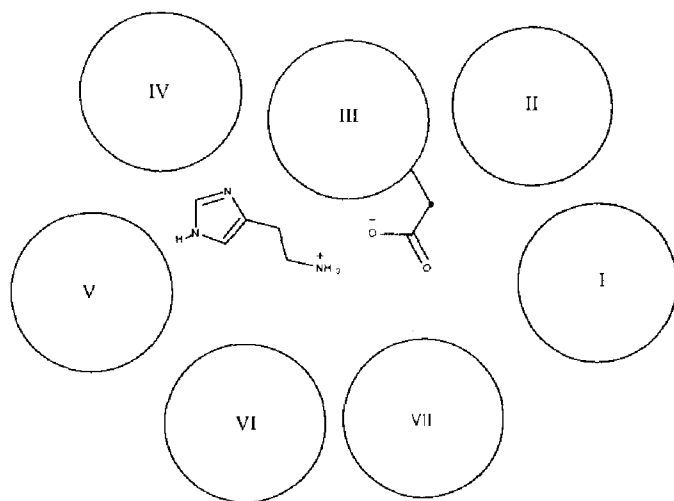


Figure 2. Schematic representation of the binding of histamine in the cavity enclosed by the seven hydrophobic transmembrane helices (viewed from the surface of the plasma membrane). The numbering and arrangement of the helices is analogous to that for the model of rhodopsin.²¹ The carboxylic acid of Asp in TMIII is shown interacting with the positively charged amino-group of histamine.

In addition to the predominant role in the binding of agonists, for some receptors this highly conserved Asp is also involved in the binding of antagonists. Therefore, the binding sites of agonists and antagonists can partly overlap with each other, *e.g.*, the aforementioned Asp in TM III of the β adrenergic and histamine H₁ receptors^{20,22} interacts with both the agonists and antagonists of these receptors.

As molecular biological data of the H₃ receptor are scarce, pharmacophore models can only be derived from receptor mapping, using the characteristics of the ligands. For H₃ ligands, the most manifest feature of the SAR is the crucial role of the 4(5)-substituted imidazole unit that is present in all potent ligands, both agonists and antagonists. Whereas additional substituents on the imidazole ring lead to dramatically reduced H₃ receptor activity, replacement of the imidazole moiety by other functional groups results in compounds with no H₃ activity at all. This essential role of the imidazole ring in these classes of agonists and antagonists implies that this moiety binds to the same receptor site and that this interaction is compulsory.

Another substructure present in all agonists and most antagonists is a basic nitrogen atom in the side chain. Assuming that the imidazole group of all agonists and antagonists binds in the same manner to the H₃ receptor, we speculate that the Asp residue in TM III (that interacts with the amino-group of the agonists, *vide supra*) is also available for binding antagonists. To investigate this hypothesis we selected propionic acid to mimic the Asp of the receptor. This procedure results in an Asp-pharmacophore complex that can be defined by the relative position of the imidazole ring of the ligands with respect to the C α and C β atoms of the aspartic acid residue (Figure 2 and Figure 6). A similar approach has been applied successfully in the development of a pharmacophore model for the H₁ receptor.²⁰

The ligands

For a review of the SAR of H₃ ligands the reader is referred to chapter 1 and to the literature.^{23,24} The H₃ agonists investigated in this study and their activities are shown in Figure 3. Recently, Sippl and co-workers predicted the biologically active conformation of H₃ agonists.²⁵ Superimposing all relevant conformations of a series of selected compounds (using (R) α , (S) β -dimethylhistamine (**2**) as a template) they reported a pharmacophore in which all imidazole rings and all protonated side chain nitrogen atoms were superimposed. In this model, histamine adopts a *gauche-trans* conformation.

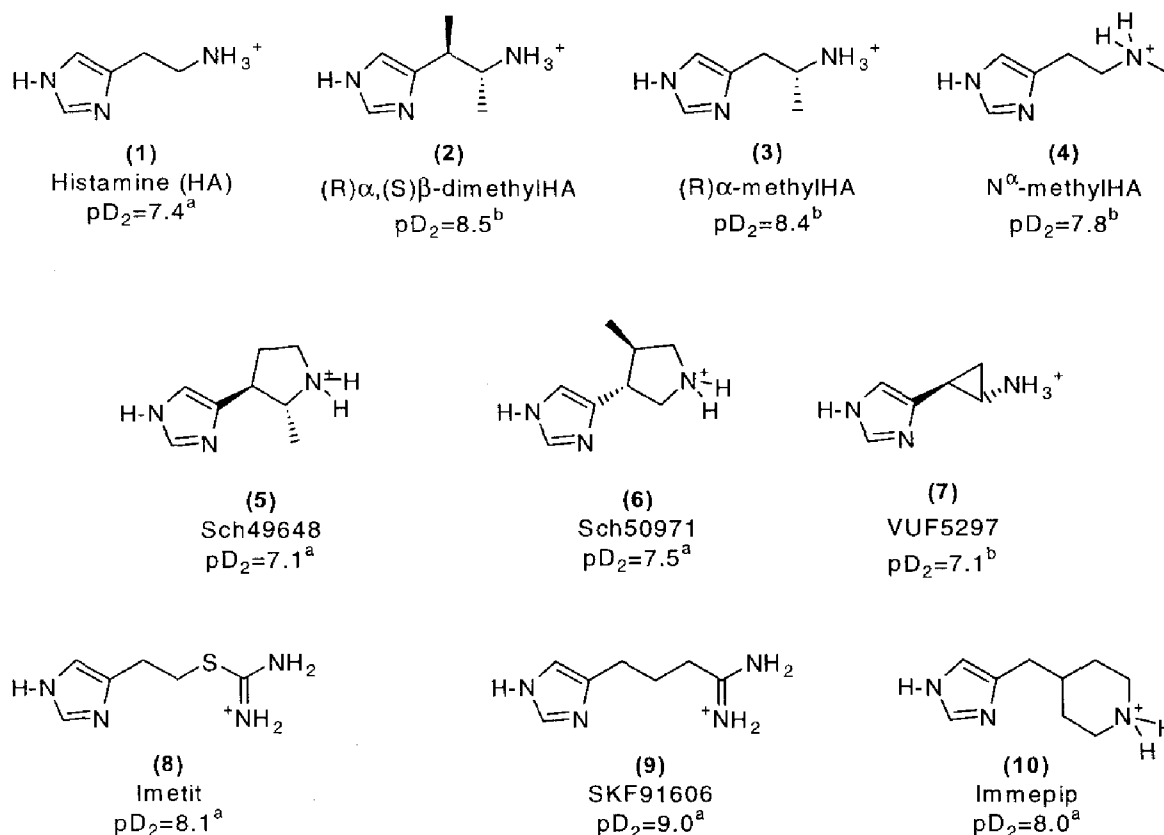


Figure 3. The H₃ agonists studied. The biological data are determined by a) evaluation of the influence of the compound on K⁺-stimulated [³H]-histamine release on rat cortex or b) evaluation of the influence of the compound on electrically evoked, cholinergic contractions of guinea pig intestine preparations.

In our study, we investigated agonists and, in addition, studied the binding modes of structurally different classes of antagonists (Figure 4). For the antagonists, the linker connecting the imidazole ring with the basic group is longer than in the agonists. Attachment of a lipophilic residue ("tail") to the basic group of antagonists can significantly increase H₃ receptor activity, indicating a hydrophobic pocket for binding these ligands. For most classes of antagonists (except for **15** and **16**), a series of derivatives has been developed to find the optimal lipophilic tail for H₃ activity. In Figure 4, one highly potent representative of each class of antagonist is shown. For most series, a comparable SAR concerning the effect of the lipophilic tail on the H₃ activity is observed (**11**, **12**, **13**, **14**). However, it has been reported that analogues of VUF 4613 (**17**) and thioperamide (**18**) have a different SAR with respect to the lipophilic tail and it has been suggested that these series of antagonists bind in a different manner.^{26,27}

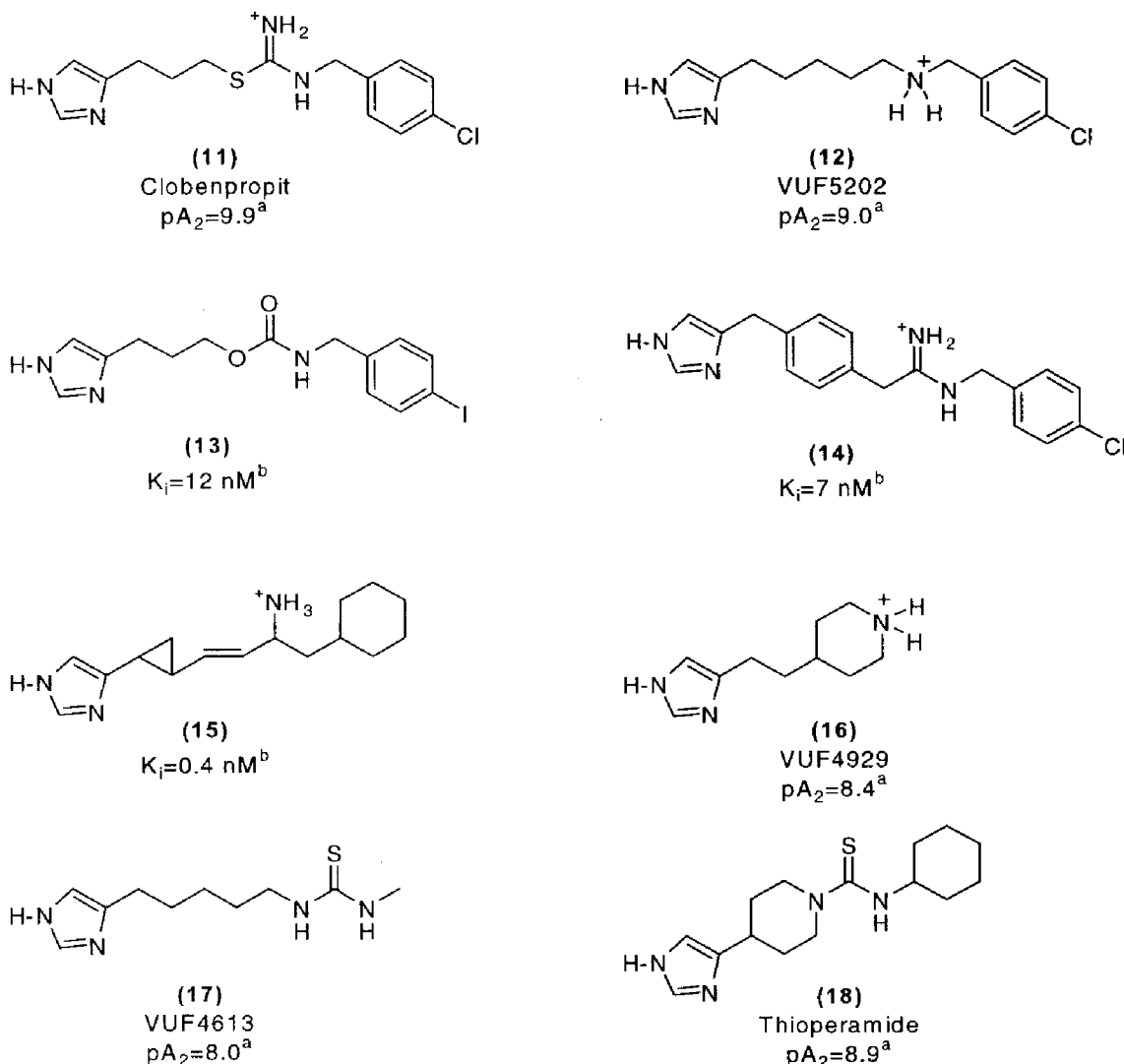


Figure 4. The H_3 antagonists studied. The biological data are determined by a) evaluation of the influence of the compound on K^+ -stimulated [3H]-histamine release on rat cortex or b) evaluation of the influence of the compound on electrically evoked, cholinergic contractions of guinea pig intestine preparations.

There are some potent H_3 antagonists that lack a basic nitrogen atom in their side chain (Figure 5). It is plausible that these ligands bind at the imidazole binding site and a lipophilic pocket, which is available for antagonist binding, without having a monopole-monopole interaction with the aspartic-acid residue. It has been shown that an ionic bond between protonated ligands and GPCRs facilitates, but is not essential for, receptor binding. For instance, it has been revealed that cationic agonists and cationic antagonists of muscarinic acetylcholine receptors form an ionic bond with the Asp in TM III, and equally potent but uncharged antagonists recognise the same binding site without interacting with this Asp.^{28,29}

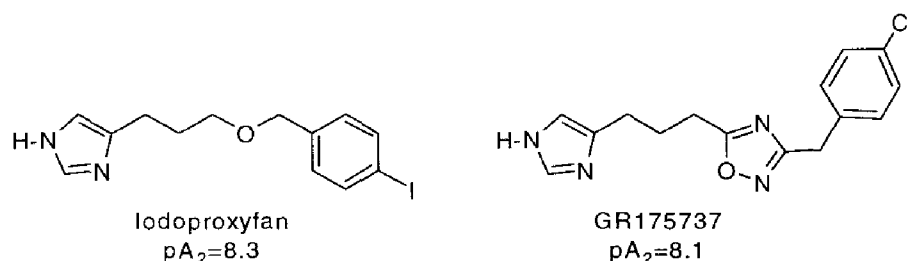


Figure 5. Histamine H₃ receptor antagonists lacking a basic nitrogen atom in their imidazole side chain.

Obviously, different antagonists can interact with a different set of receptor site points. The present study focuses on the hypothesis that the H₃ antagonists having a basic group in the imidazole side chain interact with an aspartic-acid residue known to be available for binding agonists.

Methods

Construction of the Asp-pharmacon complex

Propionic acid was selected to mimic the Asp of the receptor that interacts with the basic nitrogen atom in the side chain of the ligands. The aforementioned model for H₃ agonists derived by Sippl *et al.* was used to position the Asp with respect to histamine in the predicted bioactive conformation. The optimal interaction between the Asp (in its most stable *all trans* conformation) and histamine (in the *gauche-trans* conformation) was calculated using the methods described in the following subsections. This procedure resulted in a unique “receptor”-pharmacon complex that can be defined by the relative positions of the C α and C β atoms of the propionic acid with respect to the imidazole moiety of the ligand (Figure 6).

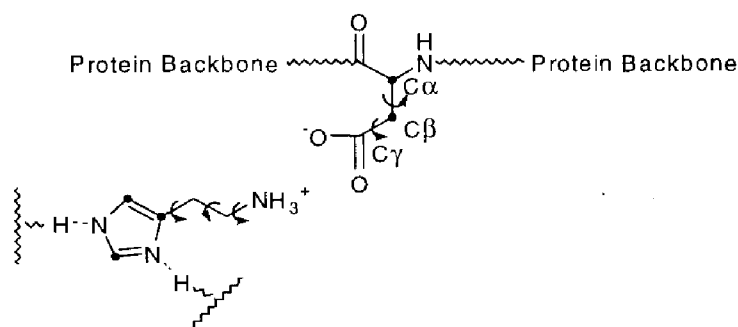


Figure 6. Schematic representation of the Asp-pharmacon complex. All ligands, agonists and antagonists, bind with their imidazole moiety at the same receptor site. Propionic acid mimics the aspartate. The fixed atoms are indicated by dots and the rotatable bonds by arrows. The C α and C β atoms of this Asp are fixed with respect to the protein backbone as altering their position can only stem from a conformational change of the α -helical transmembrane domain III. However, rotation around the C α -C β and C β -C γ bonds is allowed.

Coupling of the individual Asp-pharmacon complexes

The molecular co-ordinates of the ligands were constructed using ChemX.³⁰ The basic nitrogen atoms in the imidazole side chain were protonated, except for the nitrogen atoms of the thiourea or carbamate groups (Figure 4).³¹ Since, at present, no (experimental) indication exists regarding the bio-active tautomeric form of the imidazole moiety, we arbitrarily selected the N^{τ} -H tautomeric form (Figure 7) for all investigated compounds.

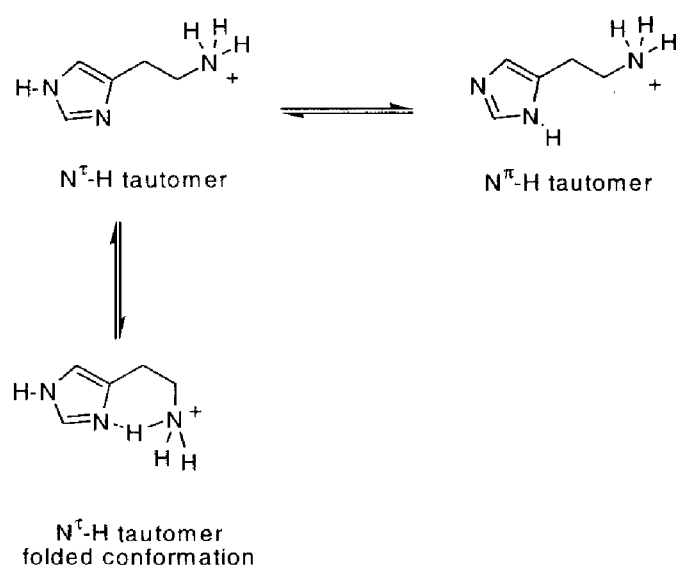


Figure 7. The two tautomeric forms of the monocation of histamine and the intramolecular hydrogen bond.

For each ligand, the Asp of the receptor was placed such that its $C\alpha$ - $C\beta$ atoms had the same relative position with respect to the imidazole ring as was described for histamine (*vide supra*). The initial coupling of the basic nitrogen atom in the side chain of the ligands to the carboxylate group was biased towards “ideal” H-bond formation.³² This was achieved by using dummy atoms (Figure 8). At the basic nitrogen of the ligand (the proton donor) a dummy atom X_a was placed at the position where the proton acceptor is expected according to a thorough analysis of X-ray data regarding H-bonds (by Ippolito and co-workers³³), *i.e.*, in the direction of the proton at a distance of 2.9 Å. At the oxygen of the carboxylate (the proton acceptor) a dummy X_d was placed at the expected position³³ of the proton donor atom, *i.e.*, in the direction of the *syn* lone-pair electrons at 2.9 Å. Subsequently, two penalty functions were used to minimise the distance between the dummies and their counter-atoms (dummy X_a and the acceptor atom *and* dummy X_d and the donor atom, respectively).

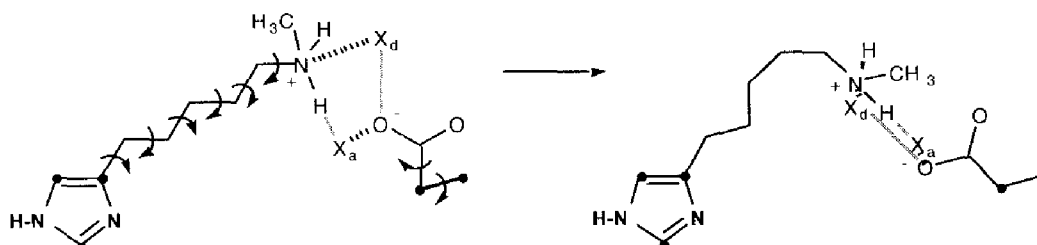


Figure 8. The initial coupling of the carboxylate and the basic nitrogen of the ligands. Fixed atoms are represented by dots. Dummies X_d and X_a represent the ideal position of the counter hydrogen-donor and hydrogen-acceptor atom, respectively. Restrain functions are represented by “|||”. During the energy minimisation procedure all torsion angles involving rotatable bonds are varied (indicated by arrows).

In the case where the “perfect” H-bond is achieved, the distance between the dummy atoms and their counter atoms is zero. These penalty functions are considered as restraint forces ($F_r = k \times \Delta r$ where k is the restraint force constant with the recommended³⁰ value of 100) during the ChemX optimisation of the ligand-Asp cluster (see Nederkoorn and co-workers for more details³²). In this optimisation procedure, the selected torsion angles are varied. The use of sufficiently high restraint forces allows overcoming rotational barriers provided that there are no serious steric hindrances. The resulting ChemX geometry, revealing in almost all cases “perfect” X-ray hydrogen bonds, merely serves (after removal of the dummy atoms) as a starting geometry for more accurate geometry optimisations (*vide infra*). For the structures containing a piperidine ring all different ring conformers were generated and used as starting geometries. To this end, MacroModel³⁴ was used for molecular mechanics conformational analysis of the different possible ring structures (using the ring closure bond option). By rotating all bonds through 360° with increments of 20°, a large number of conformations was generated. These conformations were energy optimised using the Amber force field³⁵ with the program Batchmin 2.7³⁴ in order to obtain low energy conformations.

The global minimum conformations for all ligands, of which many are highly flexible, were not determined. Therefore the underlying model remains qualitative, which is acceptable in view of the fact that the biological data stem from different pharmacological assays³⁶, indicating a large variation in activities of the agonists and antagonists. A more quantitative approach would also be hampered by the *in vacuo* formation of intramolecular hydrogen bonds between the proton of the cationic moiety in the side chain of the ligands and the basic N^π atom of the heterocycle (Figure 7). Such an intramolecular H-bond artificially lowers the

global energy of the ligands, making absolute energy differences between the biologically active conformation and the “global” minimum difficult to assess in a quantitative manner, as has been described by Nagy and co-workers.³⁷

Geometry optimisations

Because the geometries of the Asp-pharmacon complexes are strongly influenced by the formation of an intermolecular hydrogen bond, a subsequent geometry optimisation procedure was needed that could reproduce these interactions with the necessary accuracy and reliability.³⁸ Therefore, we have a density functional approach. All calculations were done at the non-local level, with density-gradient type corrections included self-consistently.³⁹ The functional of Vosko-Wilk-Nusair⁴⁰ was used for correlation and that of Becke and Perdew^{41,42} for exchange. For the geometry optimisations we used the Amsterdam Density Functional (ADF) program package⁴³ (version 2.0.3a) that runs on a parallel⁴⁴ IBM SP2 machine (a collection of powerful IBM RS/6000 processor units in one system). A triple ζ basis set with polarisation functions was applied for all atoms. The cores of C, N and O were frozen for the 1s orbital, whereas the sulfur atom was frozen up to 2p. The accuracy of the integral calculation was performed with the "A1FIT" parameter set equal to the high accuracy value of 4.0.⁴⁵ The geometry optimisations using ADF were computationally demanding and in order to keep the calculations manageable, the lipophilic tails were mimicked by a methyl group. The methyl groups give an indication of the position and orientation of the lipophilic tail of the antagonists.

Superposition of the optimised complexes

After the geometry optimisation using ADF, all the individual optimised Asp-pharmacon complexes were frozen and then superimposed (using the rigid fit command of the ChemX program package) (Figure 9). In this procedure, the imidazole rings and the C α and C β atoms were perfectly superimposed as no variance was tolerated during the ADF geometry optimisations.

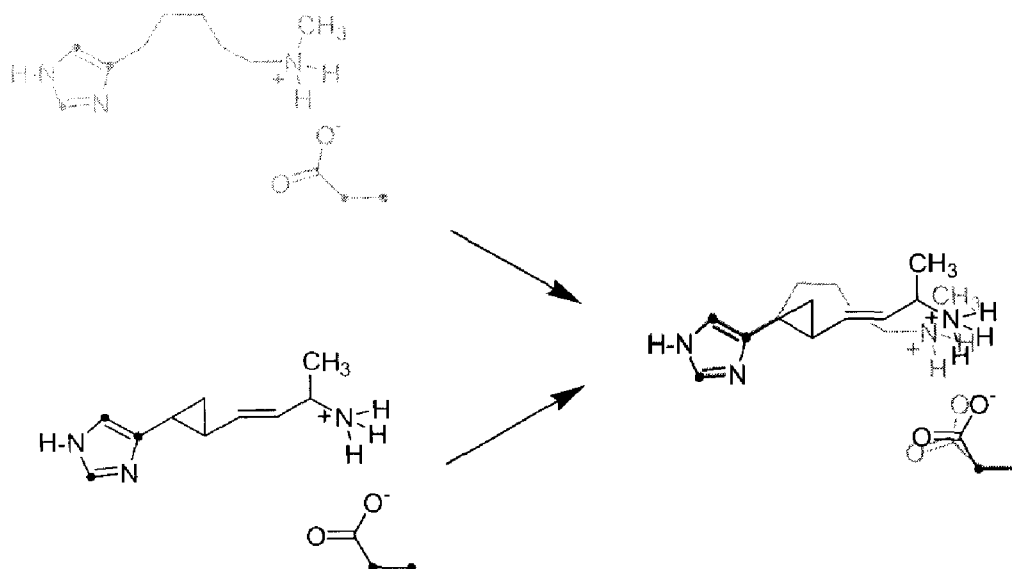


Figure 9. Schematic representation of the rigid fit of the ADF optimised pharmacon-Asp complexes. In the resulting rigid fit, the conformations and structural requirements of the complexes can be compared.

Results and discussion

Superimposing the agonist-Asp complexes (Figure 10) reveals that all agonists interact with the Asp residue in its lowest energy *all trans*-conformation. Compared to the agonist pharmacophore previously reported by Sippl *et al.*²⁵ the model presented in this chapter differs in that it is not necessary to force all basic nitrogen atoms in the side chain of the agonists to occupy the same position in space. A certain degree of positional freedom for the basic nitrogen atom allows it to obtain an optimal position with respect to the Asp. An additional advantage of our approach is that the directionality of the hydrogen bond between the basic nitrogen atom of the ligands and the receptor site point is automatically taken into account. In both Sippl's and our model, the methyl group of Sch 49648 (**5**) occupies the same region of space as the R(α)-methyl group of (R) α ,(S) β -dimethyl-histamine (**2**), while the pyrrolidine ring of **5** overlaps with the (S) β -methyl group of **2**. The relatively longer side chains of imetit (**8**) and SKF 91606 (**9**) adopt folded conformations in which they also occupy the same region of space as the (S) β -methyl group of (R) α ,(S) β -dimethylhistamine (**2**). We found that imnepip (**10**) interacts with this *all trans*-conformation of the Asp if the piperidine ring of the ligand adopts a twisted-boat conformation. In ADF geometry optimisations of the Asp-imnepip (**10**) complexes with the piperidine ring of the ligand (fixed) in other conformations, the intermolecular hydrogen bond was either lost or the resulting

conformation of the complex was in poor steric agreement with the other Asp-ligand complexes.

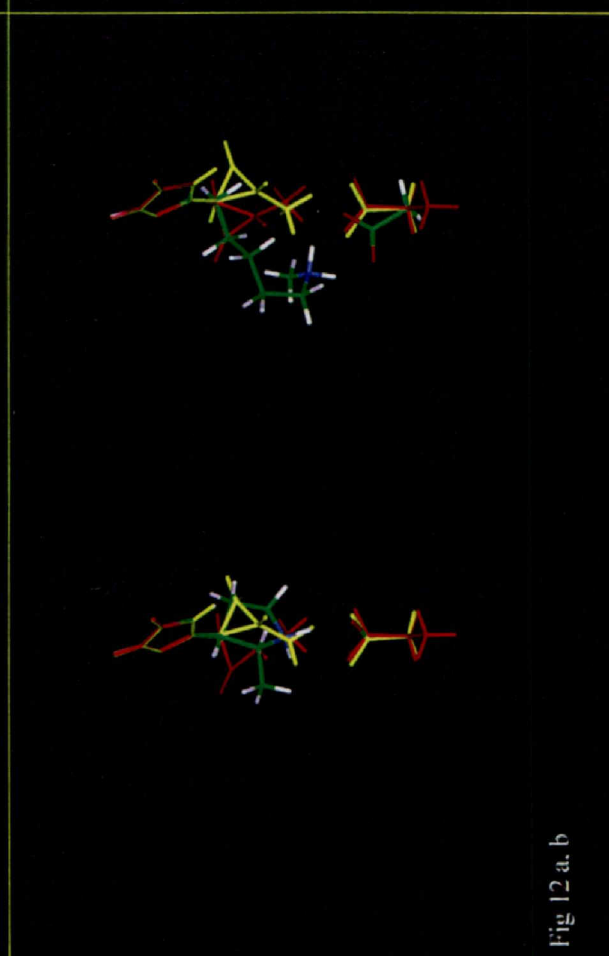
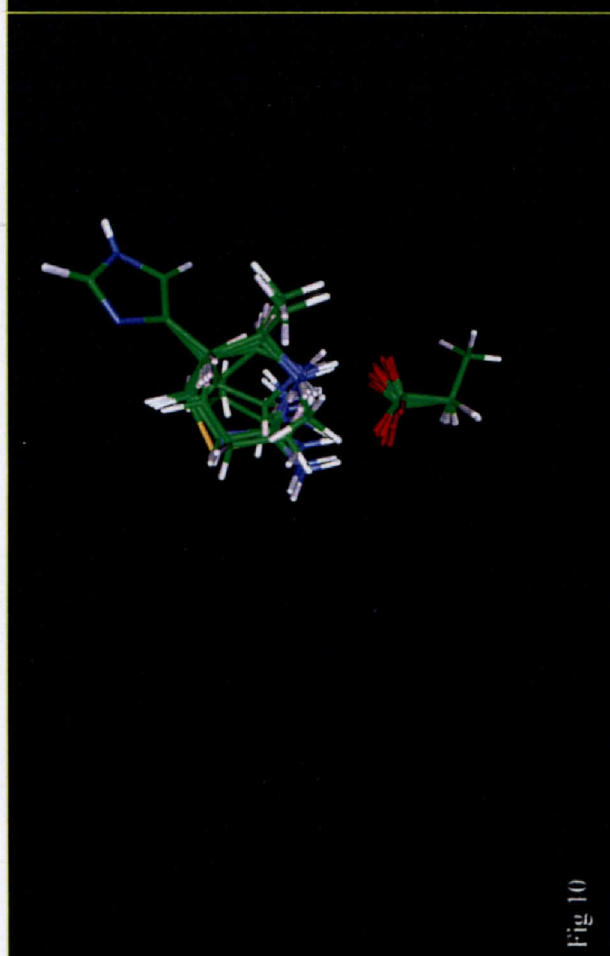
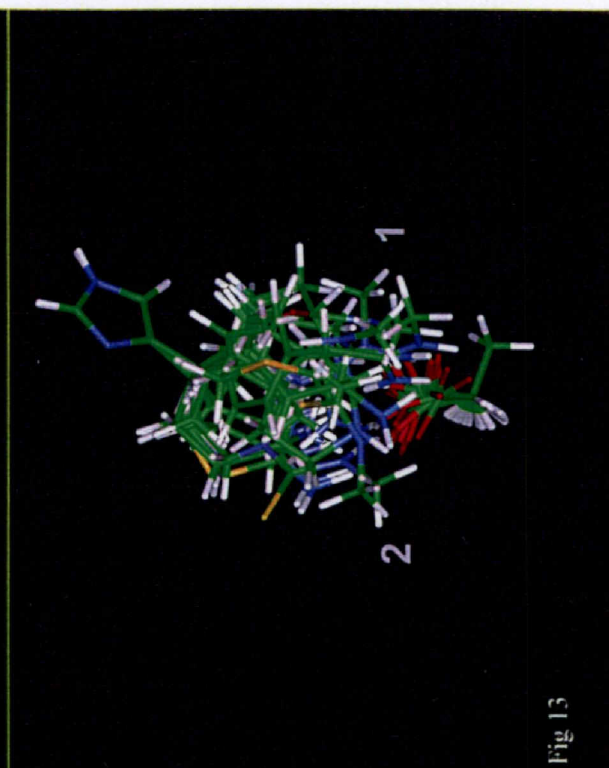
The binding mode of antagonists was studied as well. The investigated antagonists **11-18** are able to interact with the Asp that binds to the agonists. When superimposing all compounds **1-18** (Figure 11), it is noted that the position of the carboxylate is clearly different when binding to agonists or antagonists. Consequently, in the underlying model the molecular determinant accounting for agonistic vs. antagonistic activity is the conformation of the Asp. Another indication of such a molecular “switch” between agonistic vs. antagonistic activity was revealed earlier for the H₁ receptor by Ter Laak and co-workers.²⁰ In their model for H₁ antagonist binding, an H₁ agonist (2-phenyl-histamine) could be docked into the antagonist pharmacophore by altering the conformation of the interacting aspartic-acid residue in TM III. In the present study, the interaction of a large number of histamine H₃ agonists and antagonists with the flexible Asp side chain has been investigated and the position of the interacting carboxylate uniquely discriminates between agonists and antagonists. These findings again suggest an important role of the Asp in receptor stimulation.

Figure 10. The ten superimposed agonist-Asp complexes **1-10**. All imidazole rings of the ligands (upper right), all C α and all C β atoms of the Asp (bottom) occupy the same positions in 3D space for all complexes. Carbon atoms are shown in green, oxygen atoms in red, nitrogen atoms in blue, sulfur atoms in yellow and hydrogen atoms in white. These conventions are adopted throughout this paper.

Figure 11. The superimposed agonist- and antagonist-Asp complexes **1-18**. The cluster of Asp's pointing towards the upper right bind to agonists and the cluster of Asp's pointing towards the upper left bind to antagonists.

Figure 12. a) Both enantiomers of *trans*-cyclopropylhistamine (**7**), the inactive ligand VUF 5296 (red) and the active agonist VUF 5297 (yellow), give a proper interaction with the carboxylate. The structure of the eutomer is in good steric agreement with the agonist pharmacophore, illustrated by the structure of the active agonist Sch 49648 (**5**). No agonist occupies the same region of space as the inactive agonist *trans*-cyclopropylhistamine enantiomer. **b)** This region of space, occupied by the inactive ligand VUF 5296 (red) is available for binding antagonists, as illustrated by the structure of the active antagonist VUF 5202 (**12**).

Figure 13. The superimposed agonist- and antagonist-Asp complexes, alternative angle. The model reveals two distinct lipophilic pockets available for antagonist binding (indicated by 1 and 2).



To validate our model, both enantiomers of the small and rigid compound *trans*-cyclopropylhistamine were synthesised and the distinct stereoisomers were tested for their H₃ activity (described in chapter 3).⁴⁶ The enantiomers, differing significantly in pharmacological activity, give a proper interaction with the Asp in the *all trans* conformation (validating the position of this receptor site-point). However, only the structure of the eutomer (S) α ,(S) β -cyclopropylhistamine (**7**) is in good steric agreement with the agonist pharmacophore (illustrated in Figure 12a). The region of 3D space necessary to accommodate the distomer (R) α ,(R) β -cyclopropylhistamine is not accessible to agonists. However, our model does indicate that this region is available for binding antagonists (Figure 12b), thereby indicating additional conformational changes of the receptor upon stimulation.

These results have been further validated by recently published data. The cyclopropyl-containing antagonist **15** has been identified as the first stereospecific H₃ antagonist.⁴⁷ The preferred configuration of the cyclopropyl ring of the antagonist is (R) α ,(R) β -**15**, *i.e.*, the configuration that is *not* allowed for H₃ agonism. Obviously, the difference between the (impossible) binding mode of the inactive agonist (R) α ,(R) β -cyclopropylhistamine and the binding mode of the potent antagonist (R) α ,(R) β -**15** is the conformation of the interacting Asp.

As was already noted, the lipophilic tails of the antagonists were truncated to methyl groups. Our model clearly reveals two different orientations of these substituents. The methyl groups of VUF 4767 (**17**) and thioperamide (**18**) have a different position and orientation compared with the methyl groups of the other antagonists (Figure 13). These results imply the existence of two lipophilic pockets available for antagonist binding. The presence of two different lipophilic pockets explains the differences in SAR observed for the lipophilic moiety of the different classes of H₃ antagonists.

Perspectives

The model presented in this chapter reveals two distinct lipophilic pockets available for antagonist binding and therefore gives an explanation for the observed differences in the SAR of histamine H₃ receptor antagonists. The presented model has been used in a QSAR study to investigate one of these lipophilic pockets more thoroughly (see chapter 1).²⁷ The results of this QSAR study support the accuracy of the geometry of the ligand-carboxylate complexes in our H₃ ligand-binding model. Furthermore, the presented model will be used as a premise for

computationally less demanding molecular modelling studies that will enable the incorporation of antagonists that lack a basic nitrogen atom in the imidazole side chain, which were omitted in the present study.

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Chapter 5

A novel histamine H₃ receptor antagonist, 4-[2-(1H-imidazol-4-yl)-ethyl]-piperidine (VUF4929)

Abstract

A novel histamine H₃ receptor antagonist has been developed. The ligand, 4-[2-(1H-imidazol-4-yl)-ethyl]-piperidine (VUF4929), has an activity of $pA_2=8.4\pm0.1$ on the guinea pig jejunum and $pA_2=7.14\pm0.1$ on the rat cortex. Binding assays using rat cerebral cortex membranes revealed that the compound has an affinity of $pK_i=6.5\pm0.1$ when displacing the radioligand [¹²⁵I]iodophenpropit and $pK_i=7.7\pm0.1$ when displacing the radioligand [³H]N^α-methylhistamine. These remarkable discrepancies between the different pharmacological assays could be an indication for H₃ receptor heterogeneity. The biologically active conformation of the ligand was determined in molecular modelling studies. Based on this model, substitution of the nitrogen atom of the piperidine ring with various lipophilic moieties should not have major effects on the activity. To validate these theoretical findings, the basic nitrogen atom of the piperidine ring of VUF4929 was alkylated with different lipophilic moieties. The activities of the derived ligands validated the pharmacophore model.

Introduction

The first potent and selective antagonist for the H₃ receptor was thioperamide (**1**).¹ This compound contains a thioureum moiety with one nitrogen atom incorporated in a piperidine ring that links the polar thioureum moiety to the imidazole group (Figure 1) and a cyclohexyl group on the other nitrogen atom of the thioureum moiety appears to favour a high H₃ affinity (see chapter 1). In chapter 4 it is revealed that this lipophilic terminus occupies the same hydrophobic pocket of the H₃ receptor as the antagonist VUF4613 (**2**) but a different pocket to most other antagonists, *e.g.*, clobenpropit (**3**).

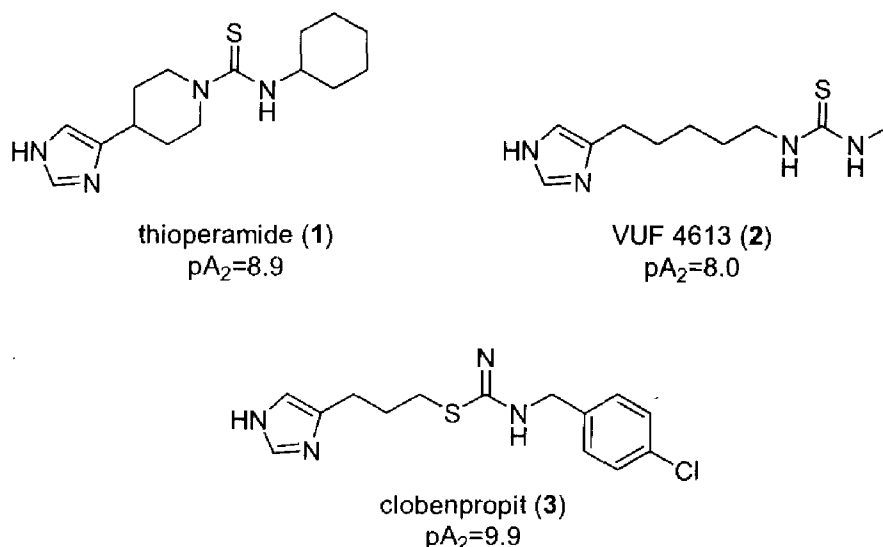


Figure 1. Three potent and selective H₃ antagonists.

The key synthetic intermediate for thioperamide, 4-(imidazol-4(5)-yl)piperine (4) (Figure 2) has only a low antagonistic activity ($pA_2=5.7$).² This compound can be considered a ring-closed derivative of VUF8326 (5) that has almost an identical H₃ activity ($pA_2=5.9$).³ A related structure, 4-[2-(1H-imidazol-4-yl)-methyl]-piperidine (immepip, 6), originally synthesised as an intermediate for the development of thioperamide (1) analogues, is a potent and selective H₃ agonist ($pD_2=8.0$).⁴ The agonistic activity of immepip (6) has been rather a surprise, as most H₃ agonists have an ethylene linker that connects the imidazole ring with a basic moiety (for a review, see chapter 1). Immepip (6) has a distinct structure and can be considered a ring-closed analogue of VUF4701 (7), a compound with antagonistic activity ($pA_2=7.1$).³ Clearly, the incorporation of the flexible butylene spacer of VUF4701 (7) in a conformationally restrained piperidine ring has major influence on both the affinity and efficacy. The modelling studies described in chapter 4 have confirmed that immepip (6) fits in the agonistic pharmacophore by adopting an energy-minimised conformation in which the piperidine ring has a twisted-boat conformation.

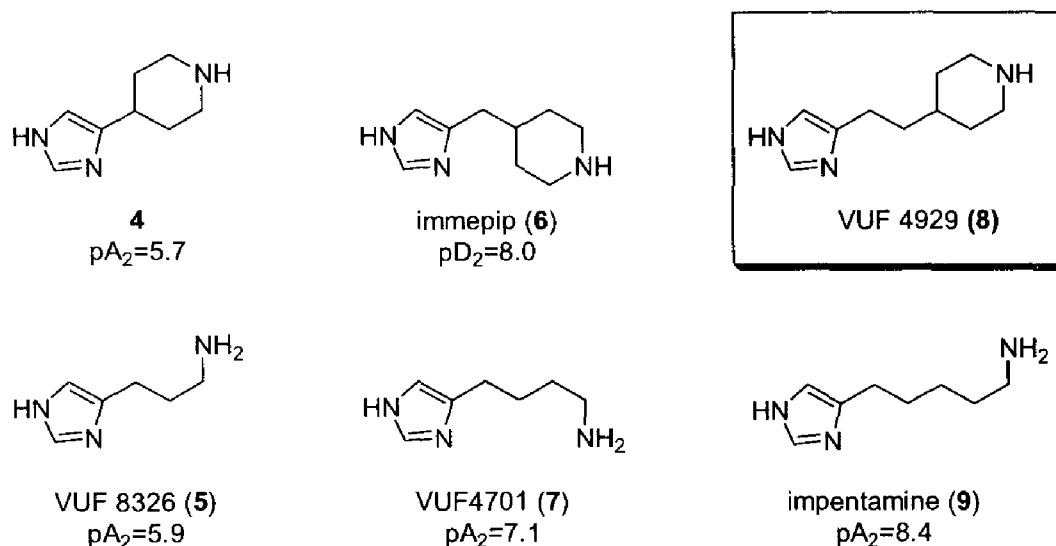
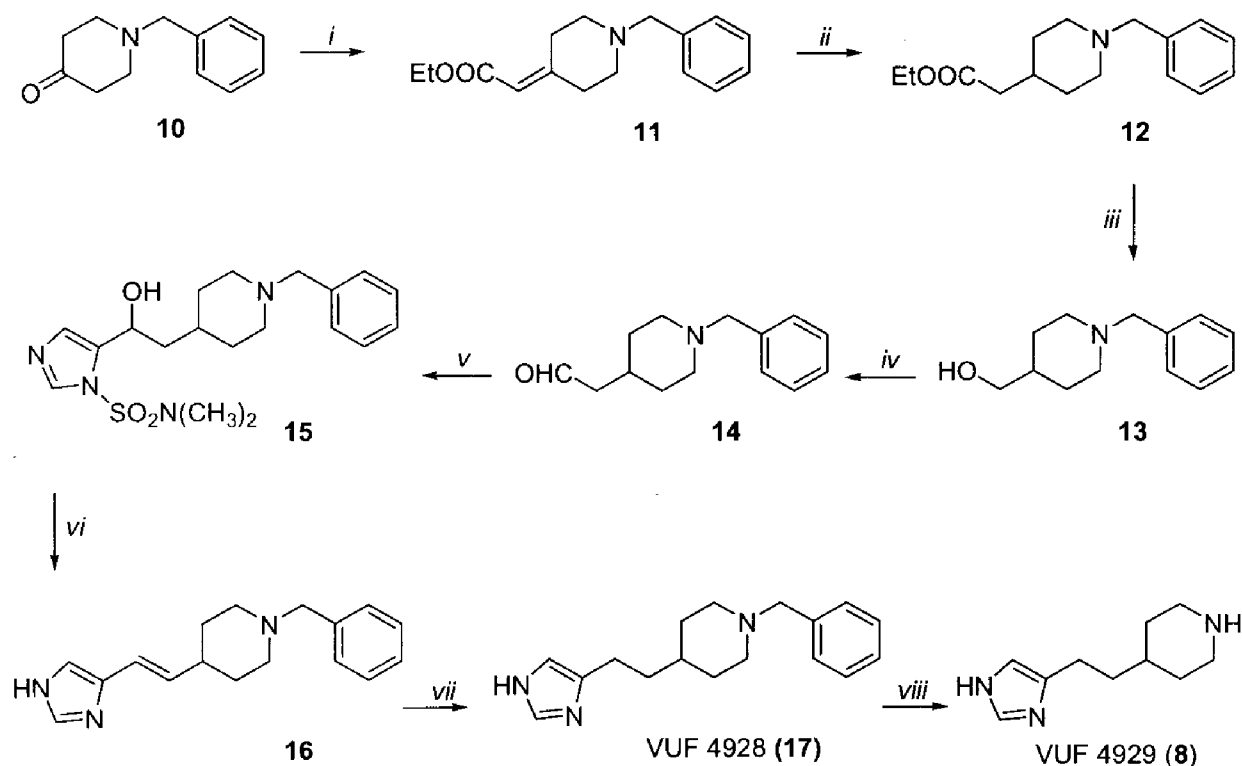


Figure 2. Structure of some H₃ receptor ligands and of the target compound VUF 4929 (7)

During the construction of the pharmacophore model, lead structure **4** was further modified. Via introduction of a longer, ethylene linker between the imidazole ring and the piperidine moiety, 4-[2-(1H-imidazol-4-yl)-ethyl]-piperidine (VUF4929, **8**) was obtained. This compound can be considered a ring-closed analogue of the potent and selective H₃ antagonist impentamine (**9**) ($pA_2=8.4$).³ In addition, a few derivatives of VUF4929 (**8**) were synthesised, in which the basic nitrogen atom of the piperidine ring was alkylated with lipophilic moieties.

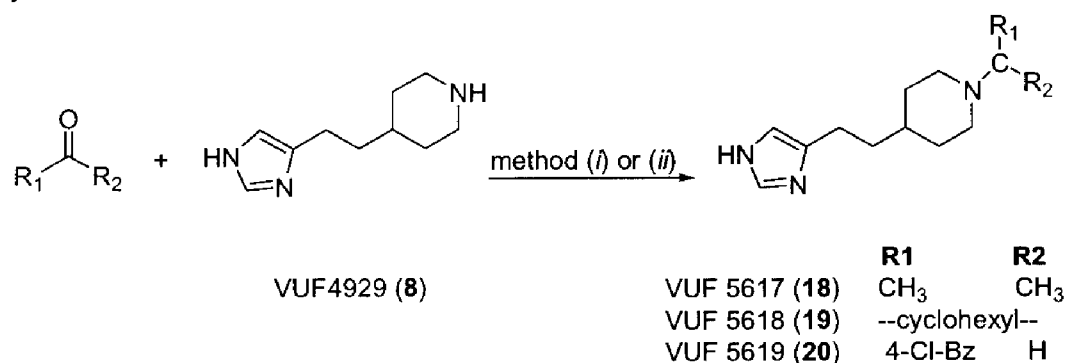
Chemistry

Commercially available 1-benzyl-4-piperidone **10** was converted into **11** via a Wadsworth-Emmons (Horner) reaction and the unsaturated double bond of **11** was reduced by catalytic hydrogenation. Reduction of the ester moiety of **12** to aldehyde **14** using diisobutylaluminum hydride (DIBALH) was only partly successful, probably because of a low quality batch of reducing agent. Therefore, the complete reaction mixture was reduced to alcohol **13** using LiAlH₄. Subsequent Swern oxidation gave pure aldehyde **14** and addition of this compound to a 5-lithiated imidazole gave intermediate **15**. Dehydration with concomitant deprotection of the imidazole ring was achieved by refluxing **15** in concentrated hydrochloric acid. Catalytic hydrogenation of **16** resulted in VUF4928 (**17**) and subsequent deprotection of the piperidine ring gave VUF4929 (**8**).



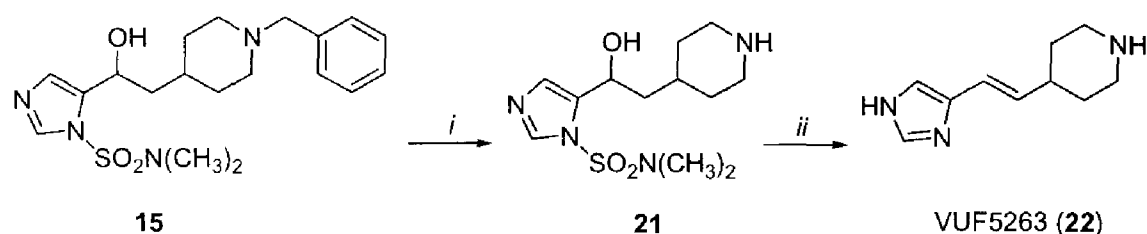
Scheme 1. Reagents used: (i) NaH, $(\text{C}_2\text{H}_5\text{O})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{CH}_3$, THF, 0°C ; (ii) 10% Pd/C, H_2 , EtOAc; (iii) DibalH, CH_2Cl_2 , -75°C ; (iii) LiAlH_4 , THF; (iv) $(\text{COCl})_2/\text{DMSO}$, CH_2Cl_2 , -55°C ; (v) 1-dimethyl-sulfamoyl-2-trimethylsilyl-5-lithio-1*H*-imidazole, THF, -65°C ; (vi) HCl, reflux; (vii) 10% Pd/C, H_2 , EtOH; (viii) 10% Pd/C, HCO_2NH_4 , MeOH.

Reductive alkylation of the amine moiety of VUF4929 (**8**) with acetone or cyclohexanone gave VUF5617 (**18**) and VUF5618 (**19**), respectively (Scheme 2). Reductive alkylation of VUF4929 (**8**) with 4-chlorobenzaldehyde and $\text{NaBH}(\text{OAc})_3$ gave VUF5619 (**20**) in good yield.



Scheme 2. Reagents used: (i) 5% Pd/C, 5% Pt/C EtOH, H_2O , CH_3COOH for **18** and **19**; (ii) $\text{NaBH}(\text{OAc})_3$, CH_2Cl_2 , EtOH, CH_3COOH for **20**.

Furthermore, a rigid analogue of VUF4929 (**8**) was synthesised (scheme 3). The piperidine ring of intermediate **15** was deprotected to give **21** and subsequent dehydration and concomitant deprotection of the imidazole ring in hydrochloric acid gave VUF5263 (**22**).



Scheme 3. Reagents used: (i) 10% Pd/C, HCO₂NH₄, MeOH (ii) 10% HCl, reflux.

Pharmacological data

The functional activities of all compounds were determined in an *in vitro* test system, based on the concentration-dependent inhibitory effect of histamine H₃ agonists on the electrically evoked contractile response of isolated guinea pig jejunum segments.⁵

The activity of VUF4929 (**8**) was also determined by measuring the release of [³H]-noradrenaline release from electrically stimulated rat cerebral cortex slices,⁶ as previously described by Alves-Rodrigues.⁷ Receptor affinities were determined using a histamine H₃ receptor binding assay on rat cortex, displacing either the radioligand [¹²⁵I]iodophenpropit or [³H]N^α-methylhistamine (for details, see the experimental section of chapter 3).⁸

Results and discussion

The compound 4-[2-(1H-imidazol-4-yl)-ethyl]-piperidine (VUF4929, **8**) is a novel and potent histamine H₃ receptor antagonist. Although the compound is structurally related to impentamine (**9**), it reveals a unique activity profile (Table 1). VUF4929 (**8**) has a pA₂=8.4±0.1 at the guinea pig jejunum and pA₂=7.1±0.1 at the rat cortex. In binding assays, the compound revealed an affinity of pK_i=6.5±0.1 when displacing the radioligand [¹²⁵I]iodophenpropit and pK_i=7.7±0.1 when displacing the radioligand [³H]N^α-methylhistamine. The remarkable discrepancies between the different pharmacological assays are an indication for the frequently suggested H₃ receptor heterogeneity.^{9,10} It has been implied that the H₃ subtypes are related to distinct functional responses, linking H_{3a} and H_{3b} receptors to H₃ receptor-mediated inhibition of histamine release and synthesis, respectively.^{11,12} Experimental pharmacological support for this

hypothesis remains limited and controversial (for a detailed discussion the reader is referred to literature^{13,14}) and for a validation of receptor heterogeneity, selective ligands will have to emerge. In this light, the remarkable activity profile of VUF4929 (**8**) is interesting, and the compound may become a valuable tool in pharmacological studies and may be a lead for the development of ligands that discriminate between the possible H₃ receptor subtypes.⁷

Table 1. H₃ receptor activity and affinity of VUF4929.

No.	Compound	pA ₂ ^a	pA ₂ ^b	pK _i ^c	pK _i ^d
8	VUF4929	8.4±0.1	7.1±0.1	6.5±0.1	7.7±0.1

^a Functional H₃ receptor assay on guinea pig jejunum.

^b Functional H₃ receptor assay on rat cortex.

^c H₃ receptor binding assay, displacing [¹²⁵I]iodophenpropit (rat brain).

^d H₃ receptor binding assay, displacing [³H]N^α-methylhistamine (rat brain).

Being structurally related to the lead compound **4** (pA₂=5.7) and imnepip (**6**; pD₂=8.0), VUF4929 (**8**; pA₂=8.4) validates earlier findings that the spacer between the imidazole ring and the cationic amino group determines affinity and efficacy (see chapter 1 and chapter 4). In the model that is described in chapter 4, the interaction of H₃ ligands with an aspartate residue (Asp) of the receptor is investigated. The agonists induce a different conformation of the Asp than the investigated antagonists, *i.e.*, the conformation of the Asp is a molecular determinant explaining efficacy. VUF4929 (**8**) was incorporated in this model, using the procedure described in detail in chapter 4. Thus, the basic nitrogen atom of the piperidine ring was coupled to the Asp and the complex was energy-minimised using the Amsterdam Density Functional (ADF) program package. The resulting, optimised VUF4929 (**8**)-Asp complex fitted the antagonistic model perfectly. In figure 3, the VUF4929 (**8**)-Asp complex is superposed with the thioperamide (**1**)-Asp complex, the VUF4613 (**2**)-Asp and the clobenpropit (**3**)-Asp complex. (The lipophilic tails of thioperamide (**1**) and clobenpropit (**3**) were truncated to methyl groups to keep the calculations manageable). These substituents give an indication of the relative position of the lipophilic tails of these ligands and, hence, an indication of the relative position of the two distinct lipophilic pockets of the H₃ receptor binding sites. The cationic amine group of VUF4929 (**8**) forms one hydrogen bond with the Asp. The other N-H bond describes a vector that is directed towards the lipophilic pocket that is used to bind the lipophilic tails of the antagonists thioperamide (**1**) and VUF4613 (**2**). Consequently, our model predicts that attachment of lipophilic moieties to the piperidine nitrogen atom of VUF4929 (**8**) should reveal a SAR that is complementary with this particular pocket. In the meantime, Windhorst and co-workers have shown that the SAR of thioperamide

(1) and derivatives is highly influenced by the unique character of the piperidine-thioureum moiety.¹⁵ Therefore, the SAR of VUF4929 (8), lacking this particular moiety, should be similar to the SAR of VUF4613 (2) as has been described by Vollinga and co-workers, *i.e.*, attachment of different lipophilic substituents to the basic moiety in the imidazole side chain does not have a major effect on the H₃ activity.¹⁶ This indicates that, although the available space in the binding site is rather limited, the receptor is able to accommodate lipophilic moieties in this region. It also suggests that no major affinity-enhancing interactions take place when a ligand occupies this pocket.

The SAR of VUF4613 (2) is different from other classes of antagonists that interact with the other lipophilic pocket, like the clobenpropit series (3), as variation of the lipophilic moieties of this latter class has a large effect on the H₃ activity (for detail see chapter 1).

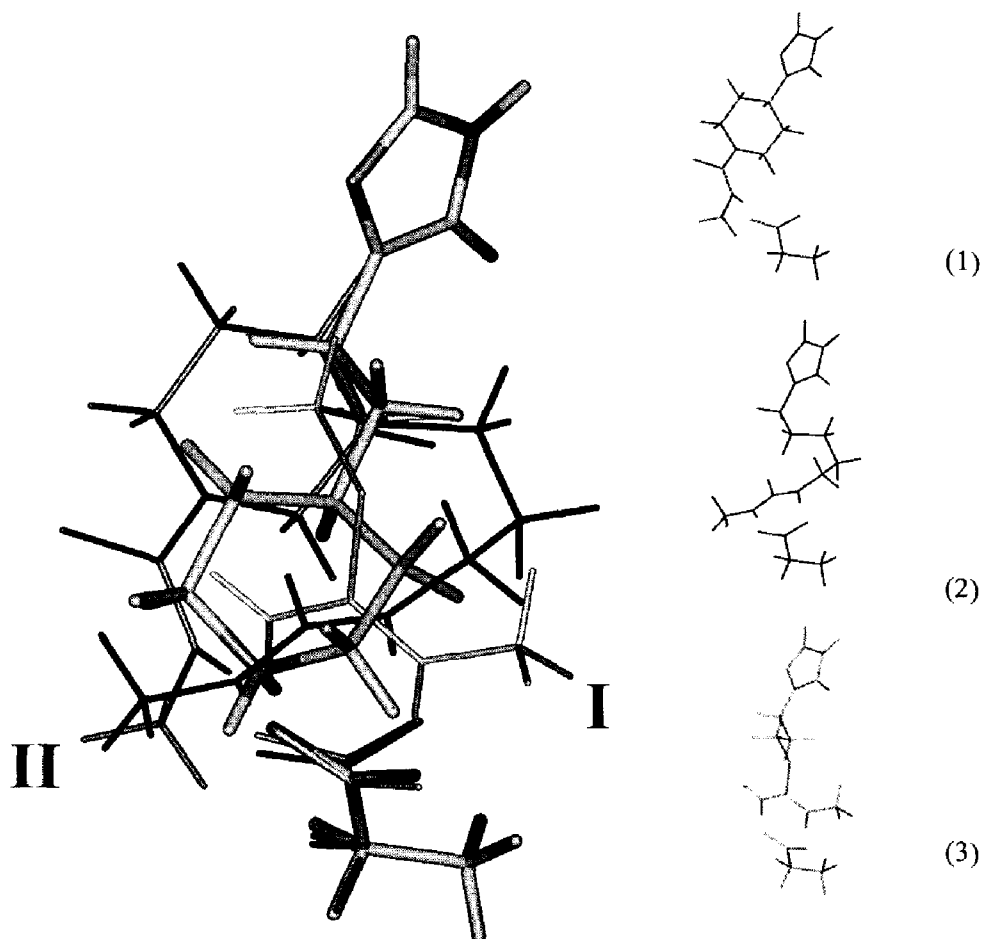
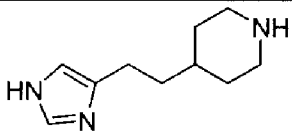
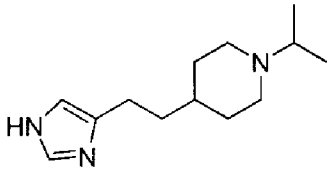
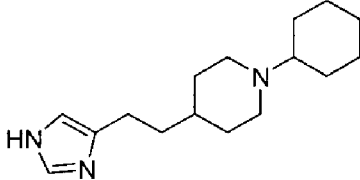
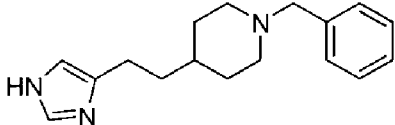
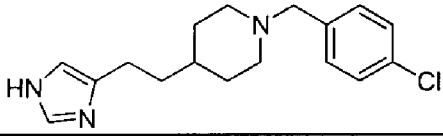
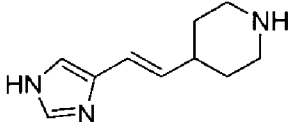


Figure 3. The bioactive conformation of selected H₃ ligands. Superposition: VUF 4929 (8) in bold, thioperamide (1) in dark grey, VUF 4613 (2) in black and clobenpropit (3) in light grey. The numbers I and II indicate the position of the lipophilic pockets. For clarity, the distinct bioactive conformation of 1, 2 and 3 are presented, in the same orientation, on the right. The lipophilic tails of 1 and 3, a cyclohexyl group and a 4-chlorobenzyl group, respectively, were truncated to methyl groups.

To investigate the SAR of derivatives of VUF4929 (**8**) a small series of compounds was synthesised and tested for H₃ activity. Compounds **17-20** have different lipophilic moieties attached to the basic nitrogen atom of the piperidine ring. All derivatives have a lower activity than VUF4929 (**8**) (Table 2). The benzyl-substituted VUF4928 (**17**; pA₂=7.5±0.1) and 4-chlorobenzyl-substituted VUF5619 (**20**; pA₂=7.8±0.1) derivatives have a slightly higher activity than the derivative VUF5617 (**18**; pA₂=7.5±0.1) and cyclohexyl-substituted derivative VUF5618 (**19**; pA₂=7.1±0.1).

Table 2. H₃ receptor activity of VUF4929 analogues.

No	Compound	Structure	pA ₂ ^a
8	VUF4929		8.4±0.1
18	VUF5617		7.4±0.1
19	VUF5618		7.1±0.1
17	VUF4928		7.5±0.1
20	VUF5619		7.8±0.1
22	VUF5263		6.4±0.3

^aFunctional H₃ receptor assay on guinea pig jejunum.

The SAR revealed by this series of compounds is different from the SAR for clobenpropit (**3**) derivatives, but comparable with the SAR of VUF4613 (**2**) derivatives (*vide supra*). E.g., replacement of the 4-chlorobenzyl group of (VUF5619, **20**) by a benzyl group (VUF4928, **17**)

does not lead to a ten fold decrease in activity as is seen for the analogous substitution for clobenpropit (see chapter 1). There are no significant differences in activity for the different alkylated analogues of VUF4929 (**8**). A similar SAR is observed for VUF4613 (**2**) and its derivatives. These results indicate that lipophilic moieties attached to the piperidine ring of VUF4929 (**8**) interact with the same hydrophobic pocket as VUF4613 (**2**), and therefore supports the theoretical model described in chapter 4.

VUF5263 (**22**) was synthesised as a rigid VUF4929 (**8**) analogue. For the construction of accurate pharmacophore models using commercially available software, conformationally restricted structures are a necessity (for a similar discussion concerning H₃ agonists, see chapter 3). All known H₃ antagonists (see chapter 1) are extremely flexible, thereby hampering the development of a more accurate H₃ antagonistic pharmacophore. VUF5263 is conformationally more restricted when compared with VUF4929 (**8**) and impentamine (**9**). Unfortunately, the rigid compound VUF5263 (**22**) is about a hundred fold less active ($pA_2=6.4\pm0.3$), indicating that the double bond forces the ligand in a conformation that cannot be accommodated by the receptor.

Conclusions

We have developed a novel and potent H₃ antagonist, 4-[2-(1H-imidazol-4-yl)-ethyl]-piperidine VUF4929 (**8**) that shows a unique H₃ receptor activity profile. The ligand reveals remarkable discrepancies in activity levels when tested in different pharmacological assays. These findings could indicate H₃ receptor heterogeneity. The biologically active conformation of VUF4929 was derived from molecular modelling studies and subsequently, these theoretical findings were validated by the synthesis of derivatives **17-20**. These derivatives, having lipophilic moieties attached to the basic nitrogen atom in the piperidine ring, are less active than the lead compound VUF4929 (**8**).

Experimental section

Chemistry

^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer with tetramethylsilane as an internal standard. Mass spectra were recorded on a Finnigan MAT-90. Solvents were purified and dried by standard procedures. 1-(*N,N*-dimethylsulfamoyl)-imidazole (**6**) was synthesised by the method described by Chadwick and co-workers, using toluene as solvent.¹⁷

(1-Benzyl-piperidin-4-ylidene)-acetic acid ethyl ester (**11**)

A suspension of THF (300 mL) and NaH (10.5 g of a 60% dispersion in oil, 0.22 mol) was cooled to 0 °C under a nitrogen atmosphere. Gradually, triethylphosphonoacetate (39.2 g, 0.18 mol) dissolved in THF (50 ml) was added. After stirring for 30 min, N-benzyl 4-piperidone (**10**) (33.1 g, 0.18 mmol) was added dropwise. The resulting suspension was stirred vigorously for 2 hours at room temperature. Water (100 mL) was added and the organic solvent was evaporated under reduced pressure and the product was extracted with dichloromethane (3 x 200 mL). The combined organic layers were washed with saturated aqueous sodium chloride (100 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexane=1/9, v/v gradient to EtOAc/hexane=1/1) to yield 27.2 g (60%) of a slightly yellow oil.

^1H NMR (CDCl_3): δ 1.3 (t, $J=7.0$ Hz, 3H), 2.20-2.26 (m, 2H), 2.38-2.43 (m, 4H), 2.90-2.93 (m, 2H), 3.51 (s, 2H), 4.10 (q, $J=7.0$ Hz, 2H), 5.63 (s, 1H), 7.11-7.32 (m, 5H).

(1-Benzyl-piperidin-4-yl)-acetic acid ethyl ester (**12**)

To **11** (29.6 g, 0.11 mol) in ethyl acetate (125 mL) was added 10% Pd/C (100 mg). The suspension was stirred for 12 hours in a hydrogen atmosphere (20 bar). The Pd/C catalyst was removed by filtration over hyflo and the volatiles were evaporated *in vacuo* to yield a light brown oil (21.7 g, 76%).

^1H NMR (CDCl_3): δ (t, $J=7.0$ Hz, 3H), 1.31-1.40 (m, 2H), 1.59-1.70 (m, 2H), 1.71-1.78 (m, 1H), 1.92-2.11 (m, 2H), 2.23 (d, $J=7.1$ Hz, 2H), 2.73-2.92 (m, 2H), 3.51 (s, 2H), 4.11 (q, $J=7.0$ Hz, 2H), 7.11-7.41 (m, 5H).

2-(1-Benzyl-piperidin-4-yl)-ethanol (**13**)

A suspension of lithiumaluminiumhydride (1.8 g, 48.1 mmol) in anhydrous THF (150 mL) was cooled to 0 °C under a nitrogen atmosphere. Gradually, **12** (12.6 g, 48 mmol), dissolved in anhydrous THF (25 ml) was added. The suspension was allowed to warm to room

temperature and subsequently refluxed for 1.5 hours. After cooling to 0 °C, water (10 mL) was added cautiously. The reaction mixture was poured into aqueous NaOH (5%, 400 mL). The organic solvent was evaporated in vacuo. The aqueous residue was extracted with diethyl ether (3 x 200 mL) and the combined organic layers were washed with saturated aqueous sodium chloride (150 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to yield a colourless oil (10.2 g, 97%).

¹H NMR (CDCl₃): δ 1.21-2.12 (m, 9H), 2.71-2.93 (m, 2H), 3.5 (s, 2H), 3.57-3.79 (m, 2H), 7.10-7.34 (m, 5H).

(1-Benzyl-piperidin-4-yl)-acetaldehyde (14)

A solution of oxalyl chloride (6.7 g, 70 mmol) in anhydrous dichloromethane (100 mL) was cooled to -60 °C under an atmosphere of dry nitrogen. A solution of DMSO (10.5 g, 0.14 mol) in anhydrous dichloromethane (50 mL) was added dropwise. After stirring for 5 min, a solution of **13** (35.81 g, 0.35 mol) in dichloromethane (100 mL) was added dropwise. After additional stirring for 15 min, triethylamine (35.8 g, 351 mmol) was added. The mixture was allowed to warm to room temperature and poured into water (150 mL). The aqueous layer was extracted with dichloromethane (4x150 mL) and the combined organic layers were washed with brine (2x100 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/methanol=9/1, v/v) to yield 7.52 g (72%) product.

¹H NMR (CDCl₃): δ (CDCl₃): δ 1.25-1.40 (m, 2H), 1.62-1.75 (m, 2H), 1.82-2.11 (m, 3H), 2.34 (d, J=7.5 Hz, 2H), 2.8-2.9 (m, 2H), 3.54 (s, 2H), 7.15-7.20 (m, 5H), 9.75 (t, J=7.5 Hz, 1H).

2-(1-Benzyl-piperidin-4-yl)-1-(1-dimethylsulfamoyl-1H-imidazol-5-yl)-ethanol (15)

A solution of 1-(*N,N*-dimethylsulfamoyl)imidazole (7.18 g, 40 mmol) in THF (200 mL) was cooled under an atmosphere of dry nitrogen to -65 °C. A solution of *n*-butyllithium in hexane (1.6 M, 23 mL, 37 mmol) was added dropwise (the internal temperature was carefully kept at -65 °C). After stirring for an additional 15 min, trimethylsilyl chloride (4.2 mL, 34 mmol) was added dropwise (the internal temperature did not exceed -65 °C). The mixture was allowed to warm to room temperature and stirred for 30 min. The solution was cooled to -65 °C and *n*-butyllithium in hexane (1.6 M, 21 mL, 33 mmol) was added dropwise. After stirring the solution for 45 min, **14** (7.52 g, 46 mmol), dissolved in THF (50 mL), was added dropwise. The mixture was allowed to warm to room temperature, stirred overnight and poured into HCl (0.5 M, 200 mL). The organic solvent was removed under reduced pressure and the residue was basified with potassium carbonate. The product was extracted with dichloromethane

(5x100 mL) and the organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product recrystallised from EA to yield a slightly yellowish solid (8.44 g, 59%). ¹H NMR (CDCl₃): δ (CDCl₃): δ 1.22-1.51 (m, 2H), 1.51-1.85 (m, 5H), 1.88-2.03 (m, 2H), 2.71-2.80 (m, 2H), 2.90 (s, 6H), 3.45 (s, 2H), 4.95 (m, 2H), 7.05 (s, 1H), 7.21-7.33 (m, 5H), 7.85 (s, 1H).

1-Benzyl-4-[2-(1*H*-imidazol-4-yl)-vinyl]-piperidine (16)

A solution of **15** (8.44 g, 20.0 mmol) in concentrated HCl (50 ml) was refluxed for 16 hours. The cooled solution was neutralised with NaOH and extracted with dichloromethane (3x100 ml). The combined organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo*. The product was isolated as yellow oil (4.81 g, 90%).

¹H NMR (CDCl₃): δ (CDCl₃): δ 1.35-1.55 (m, 2H), 1.55-1.75 (m, 2H), 1.76-1.85 (m, 1H), 1.88-2.05 (m, 2H), 2.81-2.92 (m, 2H), 3.46 (s, 2H), 5.98-6.24 (m, 2H), 6.85 (s, 1H), 7.23-7.38 (m, 5H), 7.45 (s, 1H). ¹³C NMR (CDCl₃): δ 31.7, 38.9, 53.3, 63.4, 117.8, 126.9, 128.0, 129.2, 133.3, 135.1, 137.8.

1-Benzyl-4-[2-(1*H*-imidazol-4-yl)-ethyl]-piperidine (VUF4928, 17)

To **16** (6.24 g, 23.2 mmol) in ethanol (75 mL) was added 10% Pd/C (500 mg). The suspension was stirred for 10 hours in a hydrogen atmosphere (20 bar). The Pd/C catalyst was removed by filtration over hyflo and the volatiles were evaporated *in vacuo* to yield a light brown oil (5.70 g, 98%).

¹H NMR (CDCl₃): δ 1.15-1.30 (m, 3H), 1.47-1.70 (m, 4H), 1.75-1.95 (m, 2H), 2.45 (t, J=6.3 Hz, 2H), 2.75-2.86 (m, 2H), 3.40 (s, 2H), 6.60 (s, 1H), 7.21-7.28 (m, 5H), 7.45 (s, 1H).

4-[2-(1*H*-Imidazol-4-yl)-ethyl]-piperidine (VUF4929, 8)

To **17** (5.00 g, 19.0 mmol) and ammonium formate (3.97 g, 63.0 mmol) in methanol (100 mL) was added 10% Pd/C (500 mg). The suspension was refluxed for 2 hours. The Pd/C catalyst was removed by filtration over hyflo. A solution of HBr was added (30%, 0.5 ml) and the volatiles were evaporated *in vacuo* to yield 1.50 (30%) product that was recrystallised from *iso*-propyl alcohol. Mp 207.8-208.5 °C.

¹H NMR (CDCl₃): δ (D₂O): δ 1.30-1.54 (m, 2H), 1.56-1.85 (m, 3H), 1.92-2.20 (m, 2H), 2.70-2.85 (t, J=6.5 Hz, 2H), 2.88-3.14 (m, 2H), 3.33-3.53 (m, 2H), 7.25 (s, 1H), 8.55 (s, 1H). ¹³C NMR (CDCl₃): δ (D₂O): 22.3, 29.5, 33.7, 35.1, 45.5, 116.6, 134.2, 135.0.

High resolution mass: m/z 269.1891; calculated for C₁₇H₂₃N₃: 269.1892.

N-*iso*-Propyl 4-[2-(1*H*-imidazol-4-yl)-ethyl]-piperidine (VUF 5617, 18)

To VUF4929 (**8**; free base) (0.36 g, 2.0 mmol) and acetone (4.0 ml, 54.4 mmol) in a mixture of ethanol (10.0 ml), water (5.0 ml) and acetic acid (0.11 ml) was added 5% Pd/C (50 mg) and 5% Pt/C (100 mg). The suspension was stirred for 16 hours in a hydrogen atmosphere (20 bar). The heterogeneous catalysts were removed by filtration over hyflo and the volatiles were evaporated *in vacuo*. Water (25 ml) was added and the reaction mixture was acidified with an aqueous solution of HCl (2M, 50 ml). The water layer was washed with diethyl ether (3x100 ml), neutralised with potassium carbonate and extracted with dichloromethane (3x100 ml). The combined organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo*. The free base was isolated as a slightly yellow oil (124 mg, 28%).

¹H NMR (CDCl₃): δ 1.02 (d, J=6 Hz, 6H), 1.10-1.35 (m, 3H), 1.45-1.80 (m, 4H), 1.96-2.16 (m, 2H), 2.50-2.72 (m, 3H), 2.78-2.93 (m, 2H), 6.74 (s, 1H), 7.53 (s, 1H).

N-Cyclohexyl 4-[2-(1*H*-imidazol-4-yl)-ethyl]-piperidine (VUF 5618, 19)

Analogous to the preparation of **16**, using cyclohexanone (1.96 g, 20.0 mmol).

Yield: 94 mg (18%) (oil).

¹H NMR (CDCl₃): δ 1.01-1.41 (m, 8H), 1.41-1.92 (m, 9H), 2.04-2.40 (m, 3H), 2.50-2.70 (m, 2H), 2.78-2.98 (m, 2H), 6.72 (s, 1H), 7.51 (s, 1H).

N-(4-Chlorobenzyl) 4-[2-(1*H*-imidazol-4-yl)-ethyl]-piperidine (VUF 5619, 20)

To VUF4929 (**8**; free base) (2.00 mmol) and p-chlorobenzaldehyde (0.28 g, 2.00 mmol) in mixture of dichloromethane (20 ml), ethanol (1 ml) and acetic acid (0.11 ml) was added NaBH(OAc)₃ (0.59, 3.00 mmol). After stirring the solution for eight hours under a nitrogen atmosphere, water was added (50 ml) and the reaction mixture was neutralised with an aqueous solution of NaOH (1M). The aqueous layer was washed with dichloromethane. The combined organic layers were concentrated *in vacuo* and the residue was dissolved in an aqueous solution of HCl (1M, 50 ml). The water layer was washed with diethyl ether (3x100 ml) and subsequently neutralised with an aqueous solution of NaOH (1M). The water layer was extracted with dichloromethane (3x100 ml) and the combined organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo*. ¹H NMR (CDCl₃): δ 1.11-1.32 (m, 3H), 1.51-1.82 (m, 4H), 1.85-2.03 (m, 2H), 2.51-2.70 (m, 2H), 2.74-2.90 (m, 2H), 3.41 (s, 2H), 6.70 (s, 1H), 7.01-7.29 (m, 4H), 7.51 (s, 1H).

The free base was dissolved in *iso*-propyl alcohol, and a saturated solution of oxalic acid (6.50 g, 4.00 mmol) in *iso*-propyl alcohol was added. The formed precipitate was collected by centrifugation and washed with *iso*-propyl alcohol (3x). A white solid was isolated (249 mg, 26%).

1-(1-Dimethylsulfamoyl-1H-imidazol-5-yl)-2-piperidin-4-yl-ethanol (21)

To **15** (1.70 g, 4.3 mmol) and ammonium formate (0.72 g, 11.3 mmol) in methanol (25 mL) was added 10% Pd/C (204 mg). The suspension was refluxed for 5 hours. The Pd/C catalyst was removed by filtration over hyflo. The volatiles were evaporated *in vacuo* to yield a colourless oil (1.29 g, 99%).

¹H NMR (DMSO-d₆): δ 0.92-1.12 (m, 2H), 1.42-1.73 (m, 5H), 2.33-2.53 (m, 2H), 2.90 (s, 6H), 3.29-3.41 (m, 2H), 4.84-4.98 (m, 1H), 5.10-5.45 (bs, 1H), 7.08 (s, 1H), 8.05 (s, 1H). ¹³C NMR (DMSO-d₆): 31.9, 32.3, 33.4, 37.4, 44.6, 60.2, 127.4, 136.7, 138.0 ppm.

4-[2-(1H-Imidazol-4-yl)-vinyl]-piperidine (VUF5263, 22)

A solution of **21** (1.03 g, 3.4 mmol) in concentrated HCl (50 ml) was refluxed for 5 hours. The volatiles were evaporated *in vacuo* and the product was recrystallised from EtOH to give a white powder (22 mg, 26%). Mp 192 °C (product decomposes).

¹H NMR (DMSO-d₆): δ 1.43-1.61 (m, 2H), 1.72-1.91 (m, 2H), 2.49-2.51 (m, 1H), 2.80-2.99 (m, 2H), 3.21-3.33 (m, 2H), 6.32 (d, J=16.7 Hz, 1H), 6.57 (dd, J=6.6 and 16.7 Hz, 1H), 7.65 (s, 1H), 9.08 (s, 1H). ¹³C NMR (DMSO-d₆): 32.9, 39.7, 58.3, 123.1, 124.8, 125.4, 136.7, 138.1 ppm.

Pharmacology

The functional activities of the compounds were determined according to the procedure described in chapter 2. The procedure for determining the histamine H₃ receptor affinity on rat cortical membranes with [³H]-N^α methylhistamine was also described in chapter 2. In addition, the H₃ receptor binding affinity of VUF44929 was determined with [¹²⁵I]iodophenpropit on cerebral cortex homogenates according to the procedure described by Jansen *et al.*¹⁸

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Chapter 6

Conformationally constrained analogues of 4-(1*H*-imidazol-4-yl-methyl)piperidine (immepip), as new partial agonists of the histamine H₃ receptor

Abstract

*Conformationally constrained analogues of the histamine H₃ receptor agonist immepip were synthesised. Their affinity and mode of action on the histamine H₃ receptor was determined using different pharmacological assays. In a binding assay, it was revealed that two of these ligands, 4-(1*H*-imidazol-4-yl-methylene)-piperidine and 4-[1-(1*H*-imidazol-4-yl)-vinyl]-piperidine, have moderate H₃ affinities. In a functional assay, it appeared that these compounds behave as partial agonists. Remarkably, a third derivative, (1*H*-imidazole-4-yl)-piperidine-4-yl-methanone, although structurally closely related to the other two compounds, does not have any appreciable affinity for the histamine H₃ receptor.*

Introduction

Classic pharmacological theories state that agonists of G-protein-coupled receptors activate such receptors by inducing conformational changes of the seven transmembrane helices. In this way, the extra-cellular signal is transmitted across the membrane into the cell and an intra-cellular effector system is activated. In this scheme, an antagonist blocks the receptor's binding site, preventing receptor stimulation by an agonist. However, this concept cannot explain the ability of GPCRs to activate G proteins in the absence of agonists.^{1,2} Recently, various more refined models have been proposed and currently, it is widely believed that the GPCRs are present in two receptor states, the inactive state **R**, and the active state **R***.^{3,4,5} It is assumed that only **R*** couples to the G protein. The receptor states **R** and **R*** can interconvert spontaneously (Figure 1), thereby explaining "basal" levels of second messenger generation.

In this context, ligands are classified by the level, and in which direction they shift the equilibrium between **R** and **R***. *Agonists* are compounds that stabilise the active receptor conformation **R*** ($K_A/K_{A^*} \gg 1$), thereby inducing that conformation of the receptor that couples to the G-protein. Compounds that stabilise the inactive receptor state **R**, thereby

decreasing R and, consequently, “basal” second messenger production, are called *inverse agonists* ($K_A/K_{A^*} \ll L$). *Neutral antagonists* bind to the receptor site, but have no influence on the equilibrium between R and R^* ($K_A/K_{A^*} = L$).

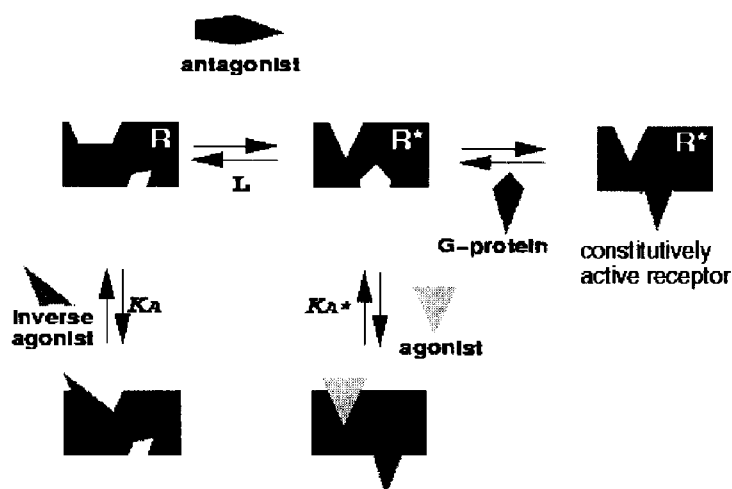


Figure 1. Simplified model for G protein activation.⁶ R represents inactive receptor state, R^* represents active receptor. The G protein can only bind to R^* . Usually, the equilibrium constant L , which is defined as $L = [R]/[R^*]$, is large, *i.e.*, the vast majority of the receptors are in the inactive state. Ligands (Lig) have different affinities for the two states of a receptor; the affinities for R and R^* are characterised by K_A and K_{A^*} , respectively ($K_A = [R][Lig]/[R \cdot Lig]$ and $K_{A^*} = [R^*][Lig]/[R^* \cdot Lig]$).

If a compound has a slight preference for binding to the active receptor conformation R^* ($K_A/K_{A^*} > L$) it would be able to change the concentration of activated receptors only slightly and display a low but detectable efficacy; these compounds are called *partial agonists*. Similar effects can be observed if a compound binds slightly better to the inactive receptor state; compounds with $K_A/K_{A^*} < L$ are called *partial inverse agonists*. The precise action of these ligands is highly dependent on environmental conditions, *e.g.*, it has been shown that the efficacy of partial agonists is shifted to the efficacy of (full) agonists under conditions with high G-protein concentration.⁷ Partial agonists are interesting potential therapeutic agents as it has been revealed that these compounds cause less receptor downregulation and desensitisation.⁸ Furthermore, because of differences in receptor-effector coupling in various tissues, partial agonists may achieve selectivity of action *in vivo*.⁹

The described model is rather dynamic, with the receptor and the ligand inducing each others conformation. A (full) agonist merely induces one unique receptor conformation: the active

receptor state **R***. As the energy differences between **R*** and **R** are generally small, very small chemical modifications can turn an agonist into a partial (inverse) agonist or even an antagonist. Full agonists of the biogenic amine receptor subfamily of GPCRs are believed to stabilise the active receptor conformation **R*** by spanning the cavity space of the receptor, interacting with a highly conserved aspartic acid residue (Asp) in transmembrane domain III (TM III) and residues in TM IV and/or TM V.^{10,11,12} The exact structural differences of the **R** and **R*** conformations (and hence the ligand binding site) are not known. However, several studies, including the work described in chapter 4, indicate that the conformation of the highly conserved Asp in TM III is different when binding to agonists and antagonists.^{13,14,15}

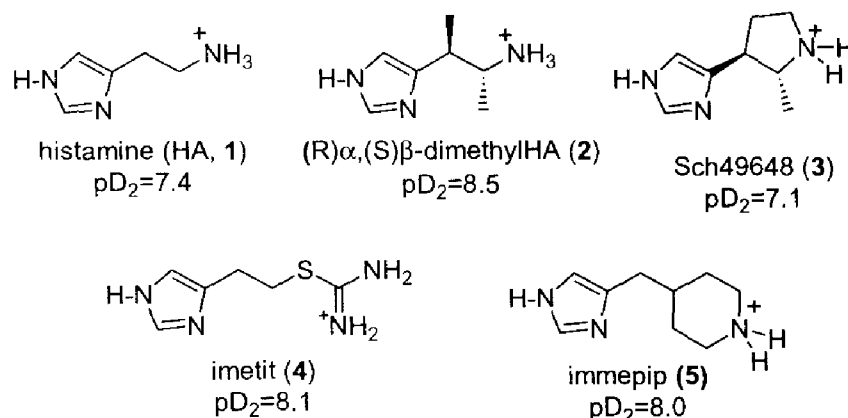


Figure 2. Five histamine H₃ receptor agonists.

In the light of the model that has a molecular descriptor for efficacy (chapter 4), the chemical modifications that convert a compound from agonist to antagonist (or *vice versa*) are very interesting. Many agonists have been described (see chapter 1) and as is illustrated in Figure 2, the structural diversity within compounds with H₃ receptor agonistic activity is limited. Interestingly, one peculiar compound, 4-(1*H*-imidazol-4-yl-methyl)-piperidine (immepip, **5**), has been described as a potent and selective H₃ agonist.¹⁶ This compound fits perfectly in the agonistic pharmacophore by adopting a folded conformation in which the piperidine ring has a twisted boat conformation (Figure 3). The incorporation of this structure was a serious challenge for the model and intensified the interest in the structure-activity relationship for this class of compounds.

In chapter 5, the development of an immepip (**5**) analogue that has an elongated linker between the imidazole moiety and the piperidine ring is described. This compound, VUF4929

(6) (Figure 3), is a potent H_3 antagonist ($pA_2=8.4$). Clearly, elongation of the linker, that connects the imidazole ring to the amino-group, by an extra methylene unit leads to complete loss of efficacy.

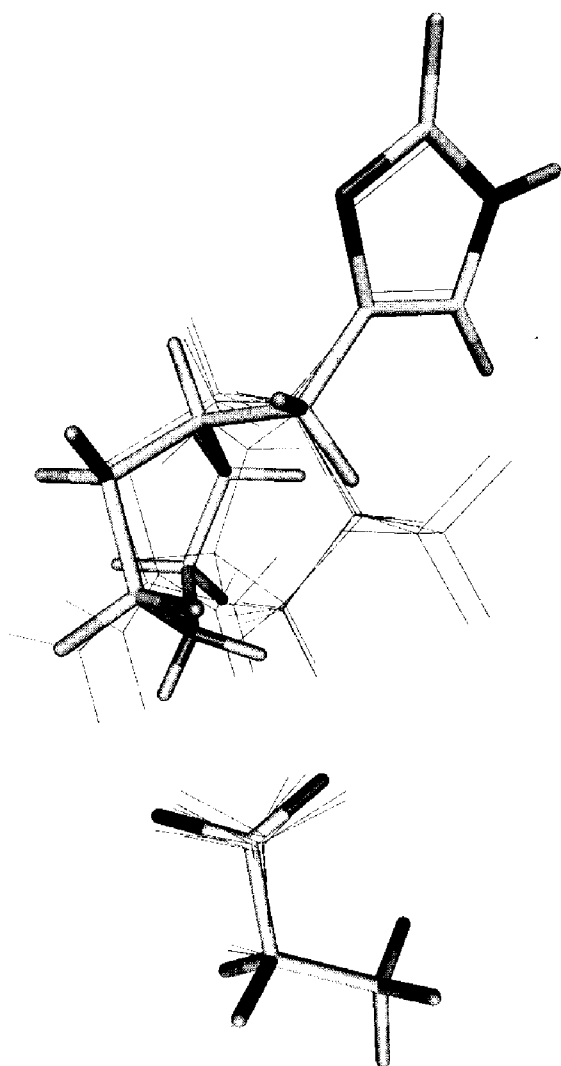


Figure 3. Some agonist-Asp complexes. Imnepip in bold, histamine, (R)α,(S)β-dimethylhistamine (2), Sch49648 (3) and imetit (4) in feint. For a detailed discussion on the agonistic pharmacophore see chapter 4.

Several other derivatives, structurally closely related to imnepip, have been mentioned in patent literature. For example, Shih and co-workers described the conformationally constrained imnepip analogue 7 (Figure 4). It was reported that this compound has a high affinity for the H_3 receptor ($pK_i=7.9$).¹⁷ Although no functional data have been published, it has been assumed that the compound is an agonist.^{18,19,20} However, the unsaturated bond in 7 hampers the compound's ability to adopt the biologically active folded conformation that is

necessary for binding to the agonistic pharmacophore. Actually, incorporation of compound **7** in the model and optimisation of the compound **7** - Asp complex, using the procedure described in chapter 4, results in an energy minimised complex that fits in the antagonistic pharmacophore. Thus, the model predicts that the rigid immepip analogue **7** is an antagonist. To verify these theoretical findings, and validate the model, the rigid immepip derivatives **8**, **9** and **10** were synthesised and tested for H_3 affinity and mode of action.

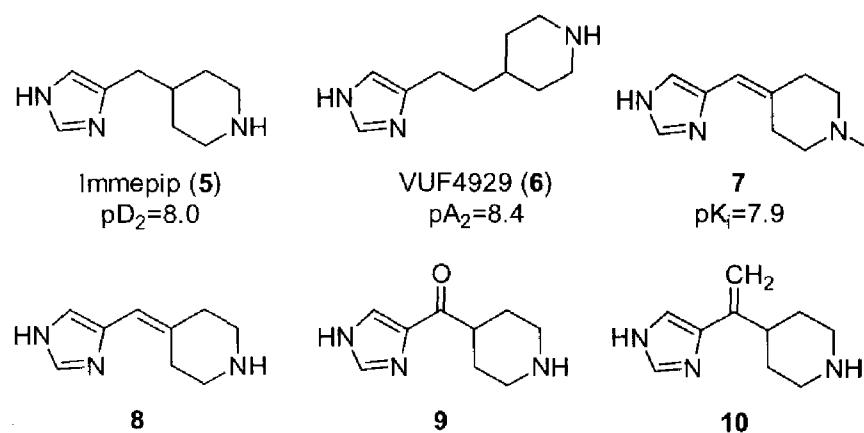


Figure 4. Immeipip, compound **2** and target compounds **8**, **9** and **10**.

The immepip derivatives **8**, **9** and **10** have an sp^2 hybridised carbon atom between the piperidine ring and the imidazole ring. This structural feature induces an extended conformation of the ligands. As with compound **7**, incorporation of compounds **8-10** in the model presented in chapter 4 yields Asp-ligand complexes that fit nicely in the antagonistic pharmacophore (Figure 5), thereby implying antagonistic activity for the ligands.

Provided that the derivatives have high antagonistic activity, these rigid compounds could serve as templates for additional molecular modelling studies of H_3 antagonists. There was need for such templates as, at the time, the flexibility of known antagonists hampered the ability of ongoing modelling studies to develop a more detailed pharmacophore model for H_3 antagonists.

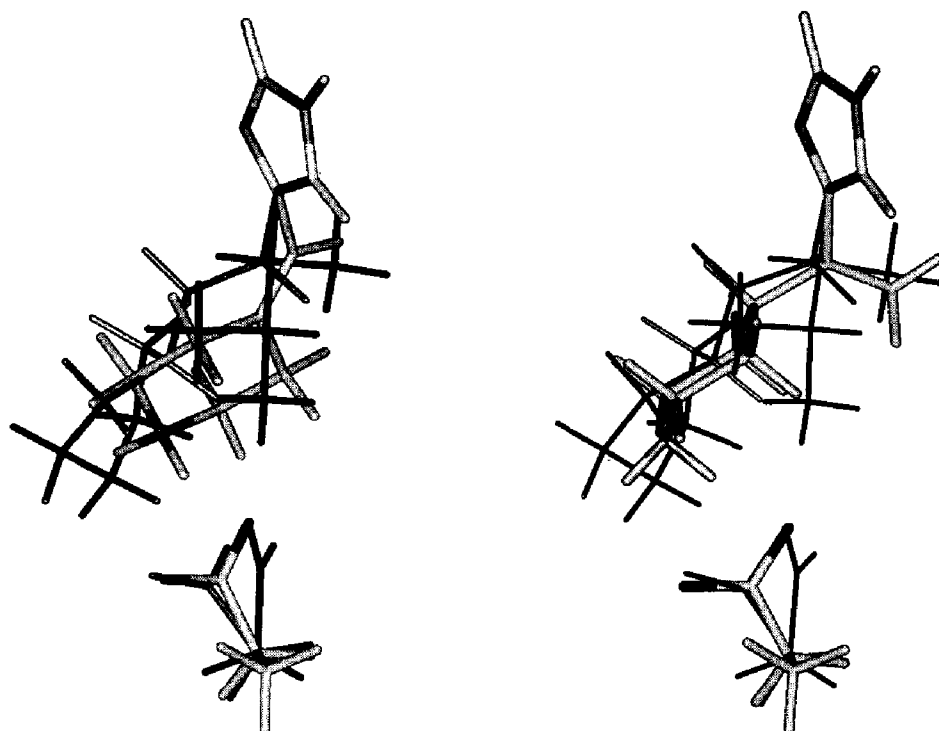
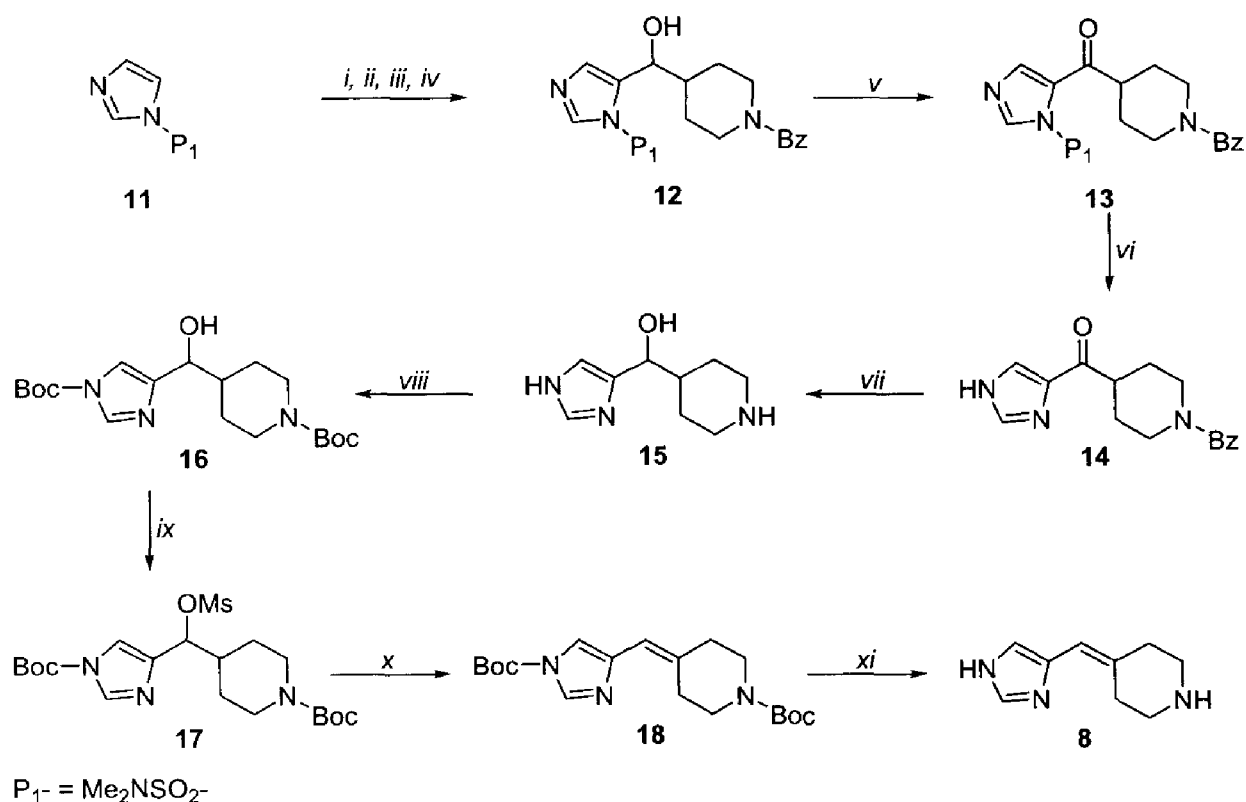


Figure 5. (a) Superimposed asp-pharmacophore complexes of ligands **8** (bold), the agonist (R) α , (S) β -dimethylhistamine (**2**) (feint, black) and the antagonist VUF 4929 (**6**) (feint, grey). Ligand **8** pulls the Asp in the conformation for binding to antagonists. Furthermore, **8** is sterically complementary to the antagonistic pharmacophore. (b) Superimposed complexes of ligand **10** (bold), (R) α , (S) β -dimethylhistamine (**2**) (feint, black) and the antagonist VUF 4929 (**6**) (feint, grey). The vinyl moiety of **10** occupies the same region of space as the S(β)-methylgroup of the agonist (R) α , (S) β -dimethylhistamine (**2**). Compound **9** has a similar binding mode to **10** (not shown).

Chemistry

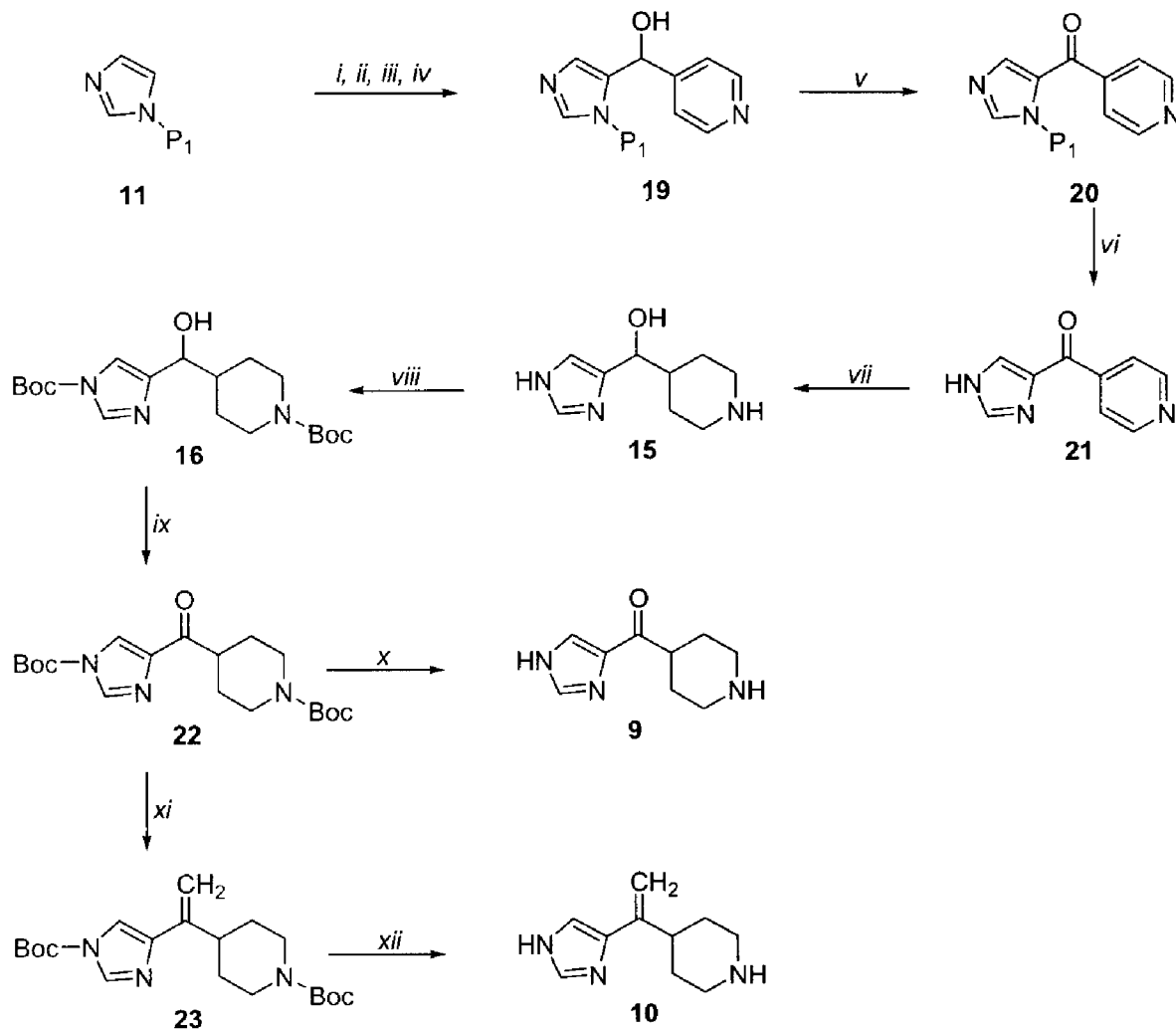
Compound **8** was prepared by the addition of 1-benzyl-piperidine-4-carbaldehyde to a 5-lithiated imidazole (Scheme 1). The use of the *N,N*-dimethylsulfamoyl protective group for the imidazole 1-position is a prerequisite for this kind of imidazole coupling reaction.²¹ The 2-position of the imidazole was protected *in situ* by a trimethylsilyl group. This group is hydrolysed rapidly during the aqueous work-up of the reaction mixture.



Scheme 1. Reagents used: (i) *n*-BuLi, THF, -70 °C; (ii) Trimethylsilyl chloride, THF; (iii) *n*-BuLi, THF, -70 °C; (iv) 1-benzyl-piperidine-4-carbaldehyde, THF; (v) (COCl)₂, CH₂Cl₂, DMSO, -55 °C; (vi) 2M HCl; (vii) Pd/C, H₂, MeOH; (viii) (Boc)₂O, Et₃N, MeOH; (ix) Ms-Cl, Et₃N, CH₂Cl₂; (x) CaCO₃, DMF, Δ; (xi) HCl (2M).

Direct conversion of alcohol **12** to the deprotected and dehydrated target compound **8** proved to be troublesome. Attempts to dehydrate alcohol **12** under acidic conditions (with concomitant deprotection of the imidazole ring) resulted invariably in tars from which no product could be isolated. Therefore, the alcohol moiety was converted into a better leaving group (*e.g.*, tosylate, mesylate, triflate). However, subsequent elimination reactions using basic conditions failed and only starting material was recovered. Furthermore, substitution of the alcohol moiety by a halogen atom, using standard literature procedure, did not give the desired halogenated compounds that might be used in subsequent elimination reactions. This lack of reactivity might be the result of steric hindrance at the reaction centre by the piperidine moiety and the *N,N*-dimethylsulfamoyl group. Removal of the *N,N*-dimethylsulfamoyl group from **12** proved to be difficult. The standard procedure for this imidazole deprotection converted **12** into tars. To facilitate deprotection of the imidazole moiety, we modified the alcohol **12** via a Swern oxidation into the keto compound **13**. Subsequently, the *N,N*-dimethylsulfamoyl group could be hydrolysed at room temperature using an aqueous solution of HCl (2M). The benzyl group on the piperidine ring was removed by catalytic

hydrogenation. During this reaction step, the ketone group was reduced to the alcohol. The basic nitrogen atoms of **15** were protected with *N*-*tert*-butoxycarbonyl (Boc) groups. According to NMR data, the imidazole ring was substituted at the more accessible N^t position (see Scheme 1). Conversion of **16** to mesylate ester **17** followed by elimination under basic conditions gave **18** and subsequent acidic hydrolysis gave **8** in high yield.



Scheme 2. Reagents used: (i) *n*-BuLi, THF, -70 °C; (ii) trimethylsilyl chloride, THF; (iii) *n*-BuLi, THF, -70 °C; (iv) 4-pyridine-carbaldehyde, THF; (v) (COCl)₂, DMSO, CH₂Cl₂, -55 °C; (vi) 2M HCl; (vii) Pd/C, H₂, MeOH; (viii) (Boc)₂O, Et₃N, MeOH; (ix); (COCl)₂, DMSO, CH₂Cl₂, -55 °C; (x) 2M HCl; (xi) methyl triphenylphosphonium bromide, potassium-*tert*-butoxide, toluene; (xii) 2M HCl.

Compounds **9** and **10** were prepared in an analogous route. In this case the lithiated 1,2-diprotected imidazole was coupled with the commercially available 4-pyridine-carboxaldehyde, resulting in compound **19**. After oxidation of the alcohol moiety, the

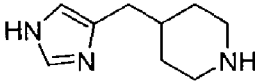
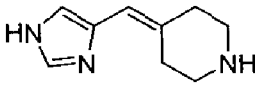
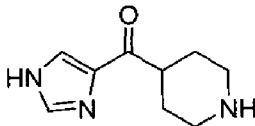
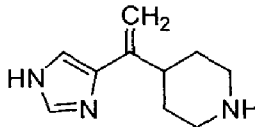
imidazole protecting group of **20** could be removed easily by an aqueous solution of HCl (2M). Reduction of the pyridine ring and the carbonyl moiety of **21**, followed by Boc-protection of the basic nitrogen atoms resulted in compound **16**. Oxidation of the alcohol group using a Swern oxidation gave intermediate **22** which was deprotected to give **9**. Conversion of intermediate **22** into **23** via a Wittig reaction and subsequent deprotection gave **10** in high yields.

Pharmacology

The affinity for the histamine H₃ receptor was determined by displacement of the radioligand [¹²⁵I]iodophenpropit using membrane homogenates of rat cerebral cortex.²²

The functional activities were determined on an *in vitro* test system, based on the concentration-dependent inhibitory effect of histamine H₃ agonists on the electrically-evoked contractile response of isolated Guinea pig jejunum segments.²³

Table 1. Histamine H₃ receptor activity of immepip and the derivatives **8**, **9** and **10**.

Compound	Structure	pK _i (nM) [n] ^a	pA ₂ [n] ^a	Efficacy α
Immepip (5)		K _H = 4.3 [8] K _L = 220 [8]	pD ₂ =8.0 [8]	1
8		16 [1]	7.4 [3]	0.5
9		> 1000 [1]	5.3 [3]	0
10		16 [1]	7.4 [3]	0.5

^a [n] Represents the number of animals used.

Discussion

Compounds **8** and **10** show moderate affinity (16 nM) for the H₃ receptor, about 4x less than immepip (**1**). In a functional assay, these compounds antagonise the effect of the selective H₃ agonist (R)- α -methylhistamine. However, the compounds are also able to activate the receptor and, therefore, have to be classified as partial agonists. We did not attempt to determine the affinities of the ligands for the different binding sites as these are difficult to quantify for partial agonists.^{7,8,9}

In the light of our model that discriminates agonists from antagonists of the H₃ ligands, the concept of partial agonism is very interesting. The geometries of the complexes that are shown in Figure 5 were derived by minimising the intra- and intermolecular energy of the Asp-pharmacon complex as described in chapter 4. The energy-minimised conformation can account for the antagonistic activity of the ligands. However, the agonistic action is not explained by these theoretical findings. The model, therefore, seems not to be able to predict partial agonism. One might have expected that a partial agonist pulls the highly conserved Asp in an intermediate agonistic/antagonistic conformation. However, this geometry is not revealed using the described optimisation procedure. Clearly, the pharmacological data presented here illustrate one of the limitations of the model presented in chapter 4.

It would be a challenge to create a model that can predict the efficacy of a ligand correctly. The recent cloning of the gene encoding for the H₃ receptor will enable the construction of a 3D model of the receptor. It would be interesting to incorporate the ligand-based model, described in chapter 4, into such a receptor model (*i.e.*, using the position of the interacting Asp as determined in the ligand-based model, and fitting it directly onto the conserved Asp in TMIII of the H₃ receptor²⁴). By investigating the binding mode of agonists and antagonists (inverse agonism has yet to be revealed for the H₃ receptor), different receptor states may be found. The binding mode of partial agonists can aid to this study, providing interesting tools to investigate the mechanism of activation of the H₃ receptor.

Compared to the affinity of compounds **8** and **10**, the low affinity of **9** is remarkable. It can be speculated that electrostatic effects caused by the polar carbonyl group of **9** may hamper binding to the receptor. Alternatively, the electron-withdrawing effect of the carbonyl group on the imidazole ring could cause the low affinity, and as such could have an influence on the putative interaction of the aromatic π -electrons of the heterocycle with the receptor. Alternatively, such a group may shift the tautomeric equilibrium of the imidazole ring to a form that is not recognised by the receptor.

Conclusions

Conformationally constrained immepip analogues have been synthesised and their histamine H₃ receptor affinity and efficacy have been determined. In a radioligand displacement study, it was revealed that compounds **8** and **10** have moderate H₃ affinity. In a functional assay, it was demonstrated that the compounds act as partial agonists. These results reveal the limitations of the pharmacophore model, as described in chapter 4, since compounds **8** and **10** can only be fitted into the antagonistic pharmacophore.

Experimental section

Chemistry.

¹H and ¹³C NMR spectra were recorded on a Bruker AC-200 spectrometer with tetramethylsilane as an internal standard. Mass spectra were recorded on a Finnigan MAT-90. Melting points were determined on a Mettler FP-5 + FP-52 apparatus and were uncorrected. GLC-Analysis was performed on a Shimadzu GC-14A equipped with an FID detector and an HP1 column (50 m x 0.31 mm). Solvents were purified and dried by standard procedures. 1-(*N,N*-Dimethylsulfamoyl)imidazole (**6**) was synthesised by the method described by Chadwick and co-workers, using toluene as solvent.²⁵

1-Benzyl-piperidine-4-carboxylic acid ethyl ester

A solution of ethyl isonipecotate (20.01 g, 127 mmol) and triethylamine (21.7 mL, 0.15 mol) in dichloromethane (2 L) was cooled to 0 °C. Benzylbromide (18.2 mL, 0.15 mol) was added dropwise. The mixture was allowed to warm to room temperature and stirred overnight. The suspension was poured into a saturated aqueous solution of sodium hydrogencarbonate (100 mL). The aqueous layer was washed with dichloromethane (2x100 mL) and the combined organic layers were washed with brine (100 mL) and dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The product was purified by column chromatography (ethyl acetate/hexane, 1/3, v/v) to give a colourless oil (27.40 g, 87%). ¹H NMR (CDCl₃): δ 1.20 (t, J= 7.0 Hz, 3H), 1.60-1.87 (m, 4H), 1.90-2.11 (m, 2H), 2.15-2.31 (m, 1H), 2.80 (m, 2H), 3.45 (s, 2H), 4.05 (q, J= 7.0 Hz, 2H), 7.08-7.18 (m, 5H).

1-Benzyl-piperidine-4-carbaldehyde

A solution of 1-benzyl-piperidine-4-carboxylic acid ethyl ester (28.18 g, 114 mmol) in toluene (200 mL) was cooled to -60 °C. A solution of diisobutylaluminium hydride in toluene (94 mL, 1.5 M, 136 mmol) was added dropwise. After completion of the reaction (3.5 hours), the mixture was quenched with saturated aqueous ammonium chloride (200 mL), neutralised with sodium carbonate (pH~10) and extracted with toluene (3x200 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. A yellowish oil was isolated (19.69 g, 85%) which was sufficiently pure for the next reaction step.

¹H NMR (CDCl₃): δ 1.22-2.34 (m, 7H), 2.45-2.70 (m, 2H), 3.41 (s, 2H), 7.23-7.31 (m, 5H), 9.60 (s, 1H).

(1-Benzyl-4-piperidin-yl)-(3-(*N,N*-dimethylsulfamoyl)-3*H*-imidazol-4-yl)-methanol (12)

A solution of 1-(*N,N*-dimethylsulfamoyl)imidazole (25.40 g, 145 mmol) in THF (300 mL) was cooled under an atmosphere of dry nitrogen to -70 °C. A solution of *n*-butyllithium in hexane (1.6 M, 100 mL, 0.16 mol) was added dropwise (the internal temperature was carefully kept at -70 °C). After stirring for an additional 15 min, trimethylsilyl chloride (20.0 mL, 0.16 mol) was added dropwise (the internal temperature did not exceed -70 °C). The mixture was allowed to warm to room temperature and stirred for 30 min. The solution was cooled to -70 °C and *n*-butyllithium in hexane (1.6 M, 94 mL, 0.15 mol) was added dropwise. After stirring the solution for 30 min, 1-benzyl-4-piperidine-carboxaldehyde (32.53 g, 0.16 mol), dissolved in CH₂Cl₂ (40 mL), was added dropwise. The mixture was allowed to warm to room temperature and poured into HCl (1M, 300 mL). The organic solvent was removed under reduced pressure and the residue was basified with potassium carbonate. The product was extracted with dichloromethane (5x100 mL) and the organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product recrystallised from IPA, resulting in a white solid (20.29 g, 37%). M.p. 136.4 °C. ¹H NMR (CDCl₃): δ 1.12-1.65 (m, 3H), 1.70-2.12 (m, 4H), 2.80-3.04 (m, 2H), 2.87 (s, 6H), 3.5 (s, 2H), 4.61 (m, 1H), 7.05 (s, 1H), 7.22-7.26 (m, 5H), 7.83 (s, 1H).

(1-Benzyl-4-piperidin-yl)-(3-(*N,N*-dimethylsulfamoyl)-3*H*-imidazol-4-yl)-methanone (13)

A solution of oxalyl chloride (3.7 mL, 42.4 mmol) and anhydrous dichloromethane (150 mL) was cooled to -55 °C under an atmosphere of dry nitrogen. A solution of DMSO (7.0 mL, 90.5 mmol) in anhydrous dichloromethane (10 mL) was added dropwise. After stirring for 5 min, a solution of **12** (13.39 g, 35.4 mmol) in dichloromethane (150 mL) was added dropwise. After

additional stirring for 15 min, triethylamine (40 mL, 300 mmol) was added. The mixture was allowed to warm to room temperature and poured into water (250 mL). The aqueous layer was extracted with dichloromethane (4x150 mL) and the combined organic layers were washed with brine (2x100 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by recrystallisation from *iso*-propylalcohol, resulting in a white solid (10.00 g, 75%). ¹H NMR (CDCl₃): δ 1.78-2.32 (m, 5H), 2.89-2.98 (m, 4H), 3.02 (s, 6H), 3.62 (s, 2H), 7.30-7.45 (m, 5H), 7.70 (s, 1H), 8.15 (s, 1H).

(1-Benzyl-4-piperidin-4-yl)-(1H-imidazol-4-yl)-methanone (14)

A solution of **13** (7.0 g, 25.0 mmol) in HCl (2M, 50 mL) was stirred for 2 hours at 60 °C. After neutralisation with NaOH, the aqueous layer was extracted with CH₂Cl₂ (3x100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. A white solid was obtained (5.1 g, 73%).

¹H NMR (DMSO-d₆): δ 1.45-1.78 (m, 4H), 1.89-2.11 (m, 2H), 2.71-2.92 (m, 2H), 3.10-3.22 (m, 1H), 3.47 (s, 2H), 7.15-7.38 (m, 5H), 7.85 (s, 1H), 7.91 (s, 1H).

(1H-Imidazol-4-yl)-piperidine-4-yl-methanol (15)

To **14** (8.0 g, 20.3 mmol) and triethylamine (11 mL, 80 mmol) in methanol (50 mL) was added 10% Pd/C (500 mg). The suspension was stirred for 12 hours in a hydrogen atmosphere (10 bar). The Pd/C catalyst was removed by filtration over hyflo and the volatiles were evaporated *in vacuo* to yield a light brown oil (4.1 g, 80%).

¹H NMR (CDCl₃): δ: 1.34-1.60 (m, 3H), 1.58-2.71 (m, 2H), 2.51-2.71 (m, 2H), 2.89-3.07 (m, 2H), 3.22-3.42 (m, 1H), 7.82 (s, 1H), 7.89 (s, 1H).

(1-(tert-Butyloxycarbonyl)-1H-imidazol-4-yl)-(1-(tert-butyloxycarbonyl)-piperidin-4-yl)-methanol (16)

To a solution of **15** (5.00 g, 19.7 mmol) and triethylamine (8.2 g, 59 mmol) in methanol (100 mL) was added *di-tert*-butyl dicarbonate (8.6 g, 39.4 mmol) and stirred overnight. The solvent was removed *in vacuo* and a saturated aqueous sodium hydrogencarbonate (50 mL) was added to the residue. The aqueous layer was washed with ethyl acetate (4x50 mL), the combined organic layers were combined, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The product was purified by column chromatography using ethyl acetate as eluent. A white solid was isolated (5.3 g, 70%). ¹H NMR (CDCl₃): δ 1.10-1.30 (m, 2H), 1.40 (s, 9H), 1.58 (s, 9H), 1.70-1.90 (m, 2H), 2.48-2.75 (m, 3H), 3.98-4.22 (m, 2H), 4.35 (m, 1H), 7.22 (s, 1H), 8.02 (s, 1H).

Methanesulfonic acid (1-(*tert*-butyloxycarbonyl)-1*H*-imidazol-4-yl)-(1-(*tert*-butyloxycarbonyl)-piperidin-4-yl)-methyl ester (17)

A solution of **16** (710 mg, 1.86 mmol) and triethylamine (0.6 mL, 4.3 mmol) in dichloromethane (10 mL) was cooled to 0 °C and methanesulfonylchloride (0.16 mL, 2.04 mmol) was added. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed *in vacuo*. The product was purified by column chromatography (ethyl acetate/triethylamine, 99/1, v/v) to give a white solid (720 mg, 84%).

¹H NMR (CDCl₃): 1.04-1.50 (m, 4H), δ 1.30 (s, 9H), 1.40 (s, 9H), 1.95-2.15 (m, 1H), 2.35-2.55 (m, 2H), 2.75 (s, 3H), 3.75-4.00 (m, 2H), 4.50 (m, 1H), 7.15 (s, 1H), 7.84 (s, 1H).

1-(*tert*-Butyloxycarbonyl)-4-(1-(*tert*-butyloxycarbonyl)-1*H*-imidazol-4-ylmethylene)-piperidine (18)

A suspension of **17** and calcium carbonate (230 mg, 2.30 mmol) in DMF (30 mL) was refluxed for 90 min. After cooling to room temperature, the suspension was filtered and concentrated *in vacuo*. The product was purified by column chromatography (ethyl acetate/methanol, 9/1, v/v) to give a white powder (184 mg, 86%).

¹H NMR (CDCl₃): δ 1.40 (s, 9H), 1.45 (s, 9H), 2.25-2.35 (m, 2H), 2.55-2.65 (m, 2H), 3.35-3.50 (m, 4H), 6.12 (s, 1H), 6.90 (s, 1H), 7.58 (s, 1H).

4-(1*H*-Imidazol-4-ylmethylene)-piperidine (8)

A solution of **18** (200 mg, 0.55 mmol) in an aqueous solution of HCl (2M, 50 mL) was stirred for 1 hour. After concentration *in vacuo*, the product was purified by recrystallisation from methanol, resulting in a white solid (57 mg, 44%). M. p. 250 °C.

¹H NMR (D₂O): δ 2.55-2.85 (m, 4H), 3.10-3.45 (m, 4H), 6.25 (s, 1H), 7.40 (s, 1H), 8.65 (s, 1H).

(3-(*N,N*-Dimethylsulfamoyl)-3*H*-imidazol-4-yl)-pyridin-4-yl-methanol (19)

The same procedure as described for **12** was followed, using pyridine-4-carbaldehyde (20.0 g, 0.19 mol) and 1-(*N,N*-dimethylsulfamoyl)imidazole (**11**) (29.8, 0.17 mol) as starting materials. The crude product recrystallised from ethyl acetate, resulting in a white solid (28.8 g, 60%).

¹H NMR (CDCl₃): δ : 2.90 (s, 6H), 6.09 (s, 1H), 6.50 (s, 1H), 7.36 (d, J=7.0 Hz, 2H), 7.85 (s, 1H), 8.42 (d, J=7.0 Hz, 2H).

(3-(*N,N*-Dimethylsulfamoyl)-3*H*-imidazol-4-yl)-pyridin-4-yl-methanone (20)

The same procedure as described for (**13**) was followed, using **19** (25.12 g, 88.97 mmol) as starting material. The product was recrystallised from *iso*-propylalcohol, resulting in a white

solid (18.95 g, 76%). M.p. 143.4 °C. ^1H NMR (CDCl_3): δ 3.15 (s, 6H), 7.50 (d, $J=7.2$ Hz, 2H), 7.65 (d, $J=7.2$ Hz, 2H), 8.20 (s, 1H), 8.80 (s, 1H).

(1*H*-Imidazol-4-yl)-pyridin-4-yl-methanone (21)

The same procedure as described for **14** was followed, using **20** (7.0 g, 25 mmol) as starting material. The product was purified by recrystallisation from acetone. A white solid was collected (6.1 g, 99%).

^1H NMR (CDCl_3): δ 8.32 (d, $J=7.5$ Hz, 2H), 8.55 (s, 1H), 9.15 (d, $J=7.5$ Hz, 2H), 9.31 (s, 1H).

(1*H*-Imidazol-4-yl)-piperidin-4-methanol (15)

To **21** (5.0 g, 20.3 mmol) and triethylamine (11 mL, 80 mmol) in methanol (50 mL) was added 10% Pd/C (500 mg). The suspension was stirred for 7 hours in a hydrogen atmosphere (10 bar). The Pd/C catalyst was removed by filtration over hyflo and the volatiles were evaporated *in vacuo* to yield a light brown oil (2.9 g, 80%).

^1H NMR (CDCl_3): δ 1.30-1.75 (m, 3H), 1.90-2.24 (m, 2H), 2.62-3.10 (m, 4H), 3.28-3.55 (m, 1H), 7.42 (s, 1H), 8.70 (s, 1H).

(1-(*tert*-Butyloxycarbonyl)-1*H*-imidazol-4-yl)-(1-(*tert*-butyloxycarbonyl)-piperidin-4-yl)-methanone (22)

The same procedure as described for **13** was followed, using **16** (2.7 g, 7.1 mmol) as starting material. The crude product was purified by column chromatography (ethyl acetate/hexane, 1/3, v/v), to give a white crystalline solid (2.56 g, 95%).

^1H NMR (CDCl_3): δ 1.42 (s, 9H), 1.62 (s, 9H), 1.30-2.00 (m, 4H), 2.85 (m, 2H), 3.50 (m, 1H), 4.15 (m, 2H), 7.98 (s, 1H), 8.04 (s, 1H).

(1*H*-Imidazole-4-yl)-piperidin-4-yl-methanone (9)

A solution of **22** (0.45 g, 1.19 mmol) was stirred in HCl (2M, 30 mL) at room temperature for 2 hours. After concentration *in vacuo*, a white solid (0.29 g, 100%) was isolated. M.p. 328 °C.

^1H NMR (D_2O): δ 1.80-2.07 (m, 4H), 2.09-2.30 (m, 2H), 3.00-3.26 (m, 2H), 3.40-3.65 (m, 1H), 8.76 (s, 1H), 9.88 (s, 1H).

1-(*tert*-Butyloxycarbonyl)-4-[1-(1-(*tert*-butyloxycarbonyl)-1*H*-imidazol-4-yl)-vinyl]-piperidine (23)

To a solution of methyl triphenylphosphonium bromide (5.2 g, 14.6 mmol) in toluene (150 mL) potassium-*tert*-butoxide (1.64 g, 14.6 mmol) was added. After stirring for half an hour at room temperature, a solution of **22** (2.76, 7.3 mmol) in toluene (80 mL) was added dropwise.

The solution was stirred for three hours and quenched with a saturated solution of sodium hydrogencarbonate (200 mL). The water layer was extracted with toluene (4x100 mL) and the combined organic layers were washed with brine (2x150 mL), dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The product was purified by column chromatography (ethyl acetate/hexane, 1/2, v/v) to yield a white solid (1.35 g, 49%). ¹H NMR (CDCl₃): δ 1.45 (s, 9H), 1.62 (s, 9H), 1.20-1.50 (m, 2H), 1.70-1.92 (m, 2H), 2.45-2.62 (m, 1H), 2.65-2.88 (m, 2H), 4.05-4.30 (m, 2H), 5.00 (s, 1H), 5.70 (s, 1H), 7.30 (s, 1H), 8.00 (s, 1H).

4-[1-(1*H*-Imidazol-4-yl)-vinyl]-piperidine (10)

A solution of **23** (0.40 g, 1.05 mmol) was stirred in HCl (2M, 30 mL) at room temperature for one hour. After concentration *in vacuo*, a white solid (0.26 g, 100%) was isolated. M.p. 271 °C. ¹H NMR (D₂O): δ 1.55-1.86 (m, 2H), 2.00-2.20 (m, 2H), 2.60-2.86 (m, 1H), 3.00-3.20 (m, 2H), 3.40-3.60 (m, 2H), 5.35 (s, 1H), 5.65 (s, 1H), 7.05 (s, 1H), 8.65 (s, 1H).

Pharmacology

The affinity for the histamine H₃ receptor was determined by displacement of the radioligand [¹²⁵I]-iodophenpropit²² using membrane homogenates of rat cerebral cortex, as described in detail by Jansen *et al.*²⁶

The functional activities of the compounds were determined on an *in vitro* test system, described by Vollinga and co-workers, that is based on the concentration-dependent inhibitory effect of histamine H₃ agonists on the electrically-evoked contractile response of isolated Guinea pig jejunum segments.²³

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Chapter 7

Synthesis and histamine H₃ receptor activity of 4-(*n*-alkyl)-1*H*-imidazoles and 4-(ω -phenylalkyl)-1*H*-imidazoles

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Abstract

*Series of 4-(*n*-alkyl)-1*H*-imidazoles and 4-(ω -phenylalkyl)-1*H*-imidazoles were prepared, with alkyl chains varying from 2-9 methylene groups and from 1-9 methylene groups, respectively. The compounds were tested for their activity on the H₃ receptor under in vitro conditions. For the 4-(*n*-alkyl)-1*H*-imidazoles the activity was found to be proportional to chain length, ranging from a pA_2 value of 6.3 ± 0.2 for 4-(*n*-propyl)-1*H*-imidazole to a pA_2 value of 7.2 ± 0.1 for 4-(*n*-decyl)-1*H*-imidazole. For the series 4-(ω -phenylalkyl)-4*H*-imidazoles an optimum in H₃ activity was found for the pentylene spacer: 4-(ω -phenylpentyl)-1*H*-imidazole has a pA_2 value of 7.8 ± 0.1 .*

Introduction

The H₃ receptor has been shown to regulate the neuronal synthesis of histamine and also regulates the release of histamine into the synaptic cleft.^{1,2} Furthermore, the H₃ receptor acts as heteroreceptor, controlling the release of other neurotransmitters, *e.g.*, dopamine, noradrenaline, serotonin and acetylcholine.³ H₃ receptors have been identified in peripheral tissues, but the highest densities of H₃ receptors were found in distinct areas of the central nervous system, *e.g.*, cortex, substantia nigra and striatum, implying the main pharmacological targets are found in the brain.⁴ It has been speculated that H₃ antagonists may provide means for treating Alzheimer's disease, narcolepsy, schizophrenia, epilepsy, sleep disturbances and obesity.⁵ Rational design of new H₃ ligands that might be useful as therapeutic agents is only feasible if the molecular structural features that are responsible for affinity and intrinsic activity (for agonists) are resolved.

The most manifest feature of the SAR of H₃ antagonists is the crucial role of the imidazole unit present in nearly all H₃ ligands⁶ (only very recently, some non-imidazole antagonists have been described^{7,8}). Another pharmacophoric element present in many H₃ antagonists is a basic nitrogen atom in the imidazole side chain. It has been suggested that this basic nitrogen atom interacts with an aspartic acid residue of the receptor.^{9,10} However, a basic group in the imidazole side chain is not essential for H₃ antagonism and several ligands have been developed that lack a basic group in their imidazole side chain.^{11,12} These compounds are thought to interact at the imidazole binding site and a putative hydrophobic pocket.⁹

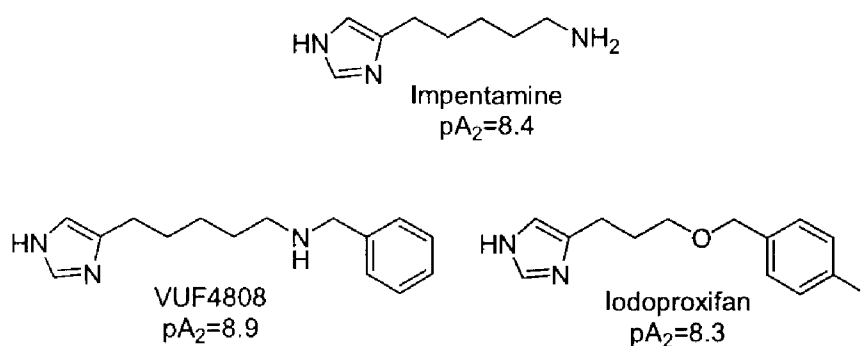


Figure 1. Structures of some H₃ receptor antagonists.

Previously, our group has synthesised histamine analogues to investigate the influence of the length of the alkyl chain (spacer), that connects the imidazole ring with an amino group, on the H₃ activity.¹³ Elongation of the ethylene spacer of histamine resulted in compounds with antagonistic activity and an optimal activity was found for the pentylene spacer, resulting in the potent and selective H₃ antagonist 4-(5-aminopentyl)-1*H*-imidazole (impentamine).¹³ An extra lipophilic group attached to the amino group significantly increased the H₃ antagonistic activity, *e.g.*, attachment of a benzyl group resulted in the potent antagonist VUF4808 (figure 1, $pA_2=8.9$).¹⁴ However, considering the activity of compounds like iodoproxifan ($pA_2=8.3$),¹⁵ it is not clear whether the amino group in the impentamine derivatives is involved in binding to a hydrogen-bonding acceptor site point of the receptor or whether the affinity of such compounds is merely caused by interaction with the imidazole binding site and a hydrophobic pocket only. The role of the lipophilic moiety of H₃ antagonists with respect to their potency has not yet been investigated systematically indeed.

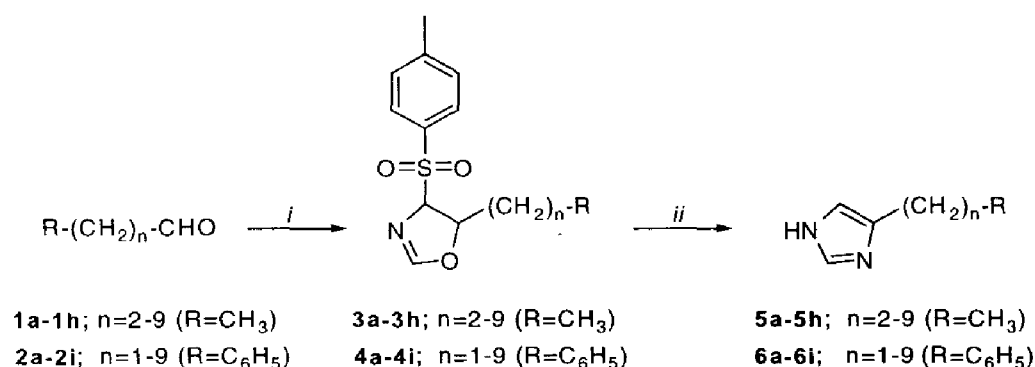
Many new H₃ antagonists have been synthesised using readily available scaffolds like urocanic acid and histamine.^{15,16} These approaches did not lead to series that allow

thorough SAR studies. However, the ongoing molecular modelling studies prompted a more detailed investigation of the role of the lipophilic moiety on H₃ antagonistic activity.⁹ Therefore, two series of compounds were synthesised to explore the SAR of the lipophilic tail of H₃ antagonists. The contribution of simple alkyl groups attached to the imidazole ring to the H₃ antagonistic activity was investigated in the first series. The effect of ω -phenyl-alkyl groups attached to the imidazole ring on the H₃ activity was studied in a second series. These new series try to fill a gap between the SAR of the antagonists with a basic moiety in the imidazole side chain and the ligands that lack this moiety, thereby enabling the evaluation of the contribution of the different molecular structural features that are responsible for affinity and antagonistic activity.

Results

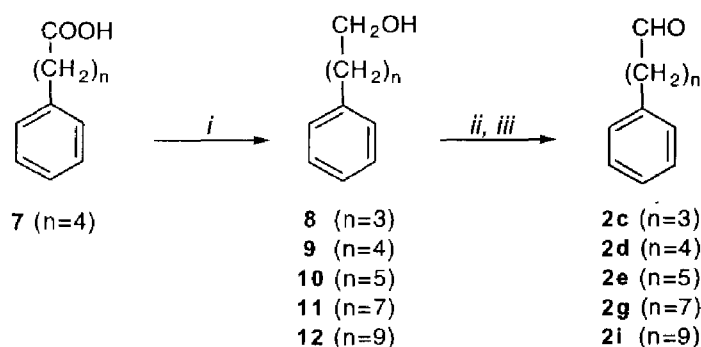
Chemistry

For the synthesis of the target compounds a general and straightforward synthetic route was employed, starting from the aldehyde precursors **1a-h** and **2a-i** (Scheme 1). These aldehydes were allowed to react with (p-tolylsulfonyl)methyl isocyanide (TosMIC), resulting in an [3+2] anionic cycloaddition.^{17,18} The 4-tosyloxazolines **3a-h** and **4a-i** precipitated from the reaction mixture in high yield and were isolated by filtration. The oxazolines were converted into the corresponding imidazoles with ammonia in ethanol.



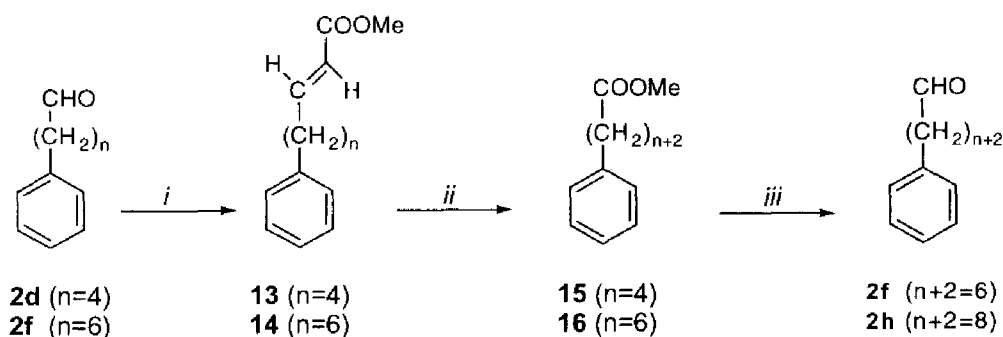
Scheme 1. Reagents used: (i) Tosmic, NaCN, EtOH, 0 °C; (ii) EtOH/NH₃; 120 °C; 15 bar.

The aldehydes used were either commercially available (aldehydes **1a-h** and **2a,b**) or prepared from readily available starting materials (**7**, **8**, **10**, **11**, **12**). Aldehydes **2c,e,g,i** were obtained by a Swern oxidation of the corresponding alcohol. Aldehyde **2d** was synthesised from the corresponding carboxylic acid **7**, via LiAlH_4 reduction and subsequent Swern oxidation (Scheme 2).



Scheme 2. Reagents used: (i) LiAlH_4 , Et_2O ; (ii) $(\text{COCl})_2/\text{DMSO}$, CH_2Cl_2 , -55°C (iii) Et_3N , CH_2Cl_2 , -55°C .

The aldehydes **2f** and **2h** were prepared by elongation of aldehyde **2d** and **2f**, respectively, via a Wadsworth-Emmons (Horner) reaction, catalytic hydrogenation and subsequent reduction of the carboxylic esters by diisobutylaluminum hydride (DIBALH) (Scheme 3).

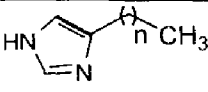


Scheme 3. Reagents used: (i) NaH , $(\text{C}_2\text{H}_5\text{O})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{CH}_3$, THF , 0°C ; (ii) 10% Pd/C , H_2 , MeOH ; (iii) DIBALH , CH_2Cl_2 , -75°C .

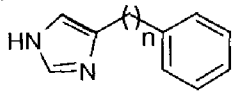
Pharmacology.

The histamine H_3 antagonistic activities of the compounds **5a-h** and **6a-i** were determined using an *in vitro* test system based on electrically evoked contractile responses of isolated guinea pig jejunum segments.¹⁹

Table 1. Histamine H₃ receptor activity of 4-(*n*-alkyl)-1*H*-imidazoles.

 $n=2-9$		
Compound	<i>n</i>	PA ₂ ^a ± SD
impentamine		8.4±0.2
5a	2	6.3±0.2
5b	3	6.7±0.1
5c	4	6.4±0.2
5d	5	6.3±0.2
5e	6	6.6±0.2
5f	7	6.8±0.1
5g	8	7.1±0.1
5h	9	7.2±0.1

^aHistamine H₃ receptor activity determined on isolated guinea pig jejunum.¹⁹**Table 2.** Histamine H₃ receptor activity of 4-(ω -phenylalkyl)-1*H*-imidazoles.

 $n=1-9$		
Compound	<i>n</i>	pA ₂ ^a ± SD
6a	1	5.8±0.1
6b	2	5.9±0.1
6c	3	6.4±0.3
6d	4	7.5±0.2
6e	5	7.8±0.1
6f	6	7.7±0.1
6g	7	7.1±0.2
6h	8	6.5±0.2
6i	9	6.1±0.2

^aHistamine H₃ receptor activity determined on isolated guinea pig jejunum.¹⁹

Discussion and conclusion

The 4-(*n*-alkyl)-1*H*-imidazoles have an antagonistic activity on the histamine H₃ receptor (Table 1) that increases with chain length from $pA_2=6.3\pm0.2$ (for 4-(*n*-propyl)-1*H*-imidazole (**5a**)) up to $pA_2=7.2\pm0.1$ (for 4-(*n*-decyl)-1*H*-imidazole (**5h**)). The remarkable activity of such simple imidazole-containing compounds illustrates the important role of the imidazole nucleus for binding to the H₃ receptor. The increasing length of the alkyl chain does apparently not hinder the binding of these flexible ligands. Entropy effects could be responsible for the increase of activity with increasing alkyl chain length. No optimum in activity was reached. Further elongation of the alkyl-chain of this series was not attempted, as the poor solubility of the higher 4-(*n*-alkyl)-1*H*-imidazoles would hamper the pharmacological testing.

For the 4-(ω -phenylalkyl)-1*H*-imidazole series, a clear optimum in H₃ antagonistic activity was found (Table 2). The low activity of 4-benzyl-1*H*-imidazole (**6a**, $pA_2=5.8\pm0.1$) is increased by elongation of the linker between the two aromatic rings, reaching an optimum in activity for the pentylene linker, 4-(5-phenyl-pentyl)-1*H*-imidazole (**6e**, $pA_2=7.8\pm0.1$). Further elongation of the linker results in a decrease of H₃ antagonistic activity, leading to the weak antagonist 4-(9-phenyl-nonyl)-1*H*-imidazole (**6i**, $pA_2=6.1\pm0.2$). During the preparation of this manuscript compounds **5g**, **6d** and **6f** were described elsewhere by Stark and co-workers.²⁰ In addition to comparable *in vitro* activity, the authors reported that these three compounds have high *in vivo* activity as well.

The series of compounds presented in our study provide additional insight into the contribution of the different molecular structural features that are responsible for H₃ activity. Thus, by comparing the activities of **6g** ($pA_2=7.1$) and VUF4808 (Figure 1, $pA_2=8.9$) it is evident that the activity of the latter is caused not only by the lipophilic moiety but that, in addition, the basic nitrogen in the side chain is involved in interaction with a hydrogen-bonding acceptor site point of the receptor.

The presented series of compounds and the SAR revealed have been essential for the construction of the pharmacophore model that is described in the next chapter (chapter 8). The compounds reveal the contribution of lipophilic moieties to the H₃ activity, and indirectly, reveal the influence of a basic group in the side chain in other classes of antagonists, *e.g.*, the impentamine series (*vide infra*). Furthermore, these compounds enable the determination of the relative position (with respect to the imidazole binding site)

of a hydrophobic pocket that is available for binding H₃ antagonists. The qualitative model described in chapter 8 is different from previous models (chapter 4) because ligands that do not have a basic nitrogen atom in the imidazole side chain can now be accommodated as the position and shape of the hydrophobic pockets of the receptor is defined. We plan to use such a model to design novel H₃ antagonists.

Experimental Section

Chemistry

¹H and ¹³C NMR spectra were recorded on a Bruker AC-200 spectrometer (unless indicated otherwise) with tetramethylsilane as an internal standard. Melting points were determined on an Electrothermal IA9200 apparatus and are uncorrected. Elemental analyses were performed by the Department of Microanalysis, Groningen University, Groningen, The Netherlands. Solvents were purified and dried by standard procedures.

4-Phenyl-butanal (2c)

A solution of oxalyl chloride (3.5 g, 28 mmol) and anhydrous dichloromethane (60 mL) was cooled to -55 °C under a nitrogen atmosphere. DMSO (4.3 g, 55 mmol), dissolved in anhydrous dichloromethane (25 mL), was added dropwise. After stirring for 5 min, 4-phenyl-butanol (**8**) (3.8 g, 25 mmol) dissolved in anhydrous dichloromethane (10 mL) was added dropwise. After additional stirring for 15 min, triethylamine (12.7 g, 125 mmol) was added. The mixture was allowed to warm to room temperature and subsequently washed with an aqueous solution of HCl (1N, 75 mL). The aqueous layer was extracted with dichloromethane (3 x 75 mL) and the combined organic layers were washed with saturated aqueous sodium chloride (75 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to yield a colourless oil.

Yield: 3.7 g (99%); ¹H NMR (CDCl₃): δ 1.95 (m, 2H), 2.44 (dt, *J*=2.0, 7.1 Hz, 2H), 2.62 (t, *J*=8.1 Hz, 2H), 7.08-7.33 (m, 5H), 9.75 (d, *J*=2.0 Hz, 1H).

5-Phenyl-pentanol (9)

A suspension of lithiumaluminiumhydride (4.3 g, 0.11 mol) in anhydrous diethyl ether (100 mL) was cooled to 0 °C under a nitrogen atmosphere. Gradually, 5-phenyl-pentanoic acid (**7**) (10.1 g, 56 mmol) was added. The suspension was allowed to warm to room temperature and stirred overnight. After cooling to 0 °C, water (100 mL) was added cautiously. The reaction mixture was poured into aqueous sulfuric acid (5%, 400 mL). The aqueous layer was extracted with diethyl ether (3 x 200 mL) and the combined organic

layers were washed with saturated aqueous sodium chloride (150 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to yield a colourless oil.

Yield: 9.3 g (100%); ^1H NMR (CDCl_3); δ 1.42 (m, 2H), 1.61 (m, 4H), 2.62 (t, $J=8.0$ Hz, 2H), 3.62 (t, $J=7.8$ Hz, 2H), 7.08-7.37 (m, 5H).

5-Phenyl-pentanal (2d)

Analogous to the preparation of **2c**, using 5-phenyl-pentanol (**9**).

Yield: 100%; ^1H NMR (CDCl_3); δ 1.64 (m, 4H), 2.45 (m, 3H), 2.63 (m, 2H), 7.00-7.29 (m, 5H), 9.68 (s, 1H).

6-Phenyl-hexanal (2e)

Analogous to the preparation of **2c**, using 6-phenyl-hexanol (**10**).

Yield: 67.0 %; ^1H NMR (CDCl_3); δ 1.35 (m, 2H), 1.60 (m, 4H), 2.33 (dt, $J=2.2, 8.0$ Hz, 2H), 2.53 (t, $J=7.4$ Hz, 2H), 6.98-7.28 (m, 5H), 9.69 (t, $J=2.2$ Hz, 1H).

Methyl 7-phenyl-2(E)-heptenoate (13)

A suspension of THF (75 mL) and NaH (1.9 g of a 60% oil dispersion, 46.7 mmol) was cooled to 0 °C under a nitrogen atmosphere. Gradually, methyl diethylphosphonoacetate (8.9 g, 42.4 mmol) was added. After stirring the reaction mixture for 30 min. 5-phenyl-pentanal (**2d**) (6.9 g, 42.4 mmol) was added dropwise. After additional stirring for 15 min, saturated aqueous ammonium chloride (100 mL) was added. The reaction mixture was extracted with diethyl ether (3 x 100 mL) and the combined organic layers were washed with saturated aqueous sodium chloride (100 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexane=1/4, v/v) to yield 8.0 g (86%) of a slightly yellow oil. R_f : 0.66 (EtOAc/hexane=1/4, v/v). ^1H NMR (CDCl_3); δ 1.41-1.72 (m, 4H), 2.28 (m, 2H), 2.60 (t, $J=6.7$ Hz, 2H), 3.65 (s, 3H), 5.80 (d, $J=16.0$ Hz, 1H), 6.94 (dt, $J=16.0, 7.2$ Hz, 1H), 7.23 (m, 5H).

Methyl 7-phenyl-heptanoate (15)

To methyl 7-phenyl-2(E)-heptenoate (**13**) (8.0 g, 37 mmol) in methanol (50 mL) was added 10% Pd/C (800 mg) and the resulting suspension was stirred overnight in a hydrogen atmosphere (10 bar). The Pd(C) catalyst was removed by filtration over hyflo and the volatiles were evaporated *in vacuo* to yield 7.4 g (91%) of a colourless oil.

^1H NMR (CDCl_3); δ 1.33 (m, 4H), 1.60 (m, 4H), 2.28 (t, $J=8.0$ Hz, 2H), 2.58 (t, $J=7.9$ Hz, 2H), 3.65 (s, 3H), 7.32-7.80 (m, 5H).

7-Phenyl-heptanal (2f)

To a cooled (-75 °C) stirred solution of methyl 7-phenyl-heptanoate (**15**) (7.4g, 33 mmol) in anhydrous dichloromethane (250 mL) was added DIBALH (35 mL of a 1 M solution in dichloromethane) dropwise. After stirring the solution for 30 min, a saturated solution of Rochelle salt (100 mL) was added. The mixture was allowed to warm up to room temperature and washed with diethyl ether. The combined organic layers were washed with an aqueous HCl solution (1M, 100 mL) and saturated aqueous sodium chloride (100 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The product was isolated as a slightly yellow oil.

Yield: 4.9 g (77%); ¹H NMR(CDCl₃); δ 1.28 (m, 4H), 1.54 (m, 4H), 2.33 (dt, *J*=8.0, 2.2 Hz, 2H), 2.52 (t, *J*=7.9 Hz, 2H), 7.02-7.28 (m, 5H), 9.69 (t, *J*=2.2 Hz, 1H).

8-Phenyl-octanal (2g)

Analogous to the preparation of **2c**, using 8-phenyl-octanol (**11**). Yield: 63.3%; *R*_f=0.67 (EtOA/hexane=1/4, v/v).

¹H NMR (CDCl₃); δ 1.30 (m, 6H), 1.59 (m, 4H), 2.39 (dt, *J*=2.4, 6.7 Hz, 2H), 2.58 (t, *J*=7.0 Hz, 2H), 7.08-7.34 (m, 5H), 9.75 (t, *J*=2.4 Hz, 1H).

Methyl 9-phenyl-2(E)-nonenoate (14)

Analogous to the preparation of **13**, using 7-phenyl-heptanal (**2f**).

Yield: 79.3 %; *R*_f=0.67 (EtOAc/hexane=1/4, v/v); ¹H NMR (CDCl₃); δ 1.28 (m, 6H), 1.53 (m, 4H), 2.12 (m, 2H), 2.52 (t, *J*=8.0 Hz, 2H), 3.63 (s, 3H), 5.73 (d, *J*=16.0 Hz, 1H), 6.90 (dt, *J*=16.0, 6.9 Hz, 1H), 7.02-7.28 (m, 5H).

Methyl 9-phenyl-nonanoate (16)

Analogous to the preparation of **15**, using methyl 9-phenyl-2(E)-nonenoate (**14**).

Yield: 91.0 %; ¹H NMR (CDCl₃); δ 1.21 (m, 8H), 1.52 (m, 4H), 2.22 (t, *J*=7.6 Hz, 2H), 2.51 (t, *J*=8.0 Hz, 2H), 3.58 (s, 3H), 7.02-7.25 (m, 5H).

9-Phenyl-nonanal (2h)

Analogous to the preparation of **2f**, using methyl 9-phenyl-nonanoate (**16**).

Yield: 97%; ¹H NMR (CDCl₃); δ 1.22 (m, 8H), 1.54 (m, 4H), 2.35 (t, *J*=8.2 Hz, 2H), 2.53 (t, *J*=7.6 Hz, 2H), 6.99-7.28 (m, 5H), 9.70 (s, 1H).

10-Phenyl-decanal (2i)

Analogous to the preparation of **2c**, using 10-phenyl-decanol (**12**). The crude yellow coloured product was purified by column chromatography (EtOAc/hexane=1/4, v/v) to give

95% of a colourless oil.

R_f: 0.69 (EtOAc/Hexane=1/4, v/v); ¹H NMR (CDCl₃): δ 1.27 (m, 10H), 1.60 (m, 4H), 2.40 (dt, J= 2.4, 6.8 Hz, 2H), 2.59 (t, J= 8.2 Hz, 2H), 7.08-7.33 (m, 5H), 9.75 (t, J=2.4 Hz, 1H).

General Procedure for the preparation of 5-(*n*-alkyl)-4-tosyl-2-oxazolines and 5-(ω-phenylalkyl)-4-tosyl-2-oxazolines.

To a stirred suspension of tosylmethyl isocyanide (20.0 mmol) and the corresponding aldehyde (20.5 mmol) in absolute ethanol (200 mL) at 0 °C was added finely powdered sodium cyanide (30 mg, 1.0 mmol). For a moment the reaction mixture became clear followed by precipitation of the product. Ten minutes after the addition of sodium cyanide, the suspension was filtered. The product was washed with ether/hexane (20 mL, 1/1, v/v) and dried in vacuo.

5-(*n*-Propyl)-4-tosyl-2-oxazoline (3a)

Yield: 83.3 %; mp 110.5-111.6 °C; ¹H NMR (CDCl₃): δ 0.96 (t, J=7.3 Hz, 3H), 1.48 (m, 2H), 1.63 (m, 2H), 2.43 (s, 3H), 4.74 (d, J=5.4 Hz, 1H), 5.07 (q, J=5.4 Hz, 1H), 6.94 (s, 1H), 7.37 (d, J=8.0 Hz, 2H), 7.80 (d, J=8.0 Hz, 2H).

5-(*n*-Butyl)-4-tosyl-2-oxazoline (3b)

Yield: 77.3 %; mp 101.1-103.0 °C; ¹H NMR (CDCl₃): δ 0.89 (t, J=7.00 Hz, 3H), 1.38 (m, 4H), 1.68 (m, 2H), 2.43 (s, 3H), 4.72 (d, J=6.00 Hz, 1H), 5.02 (q, J=6.00 Hz, 1H), 6.95 (s, 1H), 7.35 (d, J=7.60 Hz, 2H), 7.80 (d, J=7.60 Hz, 2H).

5-(*n*-Pentyl)-4-tosyl-2-oxazoline (3c)

Yield: 76.7 %; mp 117.1-118.3 °C; ¹H NMR (CDCl₃): δ 0.88 (t, J=7.00 Hz, 3H), 1.31 (m, 6H), 1.64 (m, 2H), 2.42 (s, 3H), 4.73 (d, J=6.00 Hz, 1H), 5.04 (q, J=6.00 Hz, 1H), 6.94 (s, 1H), 7.34 (d, J=8.00 Hz, 2H), 7.80 (d, J=8.00 Hz, 2H).

5-(*n*-Hexyl)-4-tosyl-2-oxazoline (3d)

Yield: 87.2 %; mp 104.5-105.6 °C; ¹H NMR (CDCl₃): δ 0.86 (t, J=7.00 Hz, 3H), 1.25 (m, 8H), 1.64 (m, 2H), 2.43 (s, 3H), 4.74 (d, J=6.00 Hz, 1H), 5.04 (q, J=6.00 Hz, 1H), 6.95 (s, 1H), 7.37 (d, J=7.40 Hz, 2H), 7.80 (d, J=7.40 Hz, 2H).

5-(*n*-Heptyl)-4-tosyl-2-oxazoline (3e)

Yield: 92.7 %; mp 118.2-119.0 °C; ¹H NMR (CDCl₃): δ 0.85 (t, J=6.67 Hz, 3H), 1.20 (m,

10H), 1.65 (m, 2H), 2.44 (s, 3H), 4.73 (d, *J*=6.70 Hz, 1H), 5.04 (q, *J*=6.70 Hz, 1H), 6.95 (s, 1H), 7.36 (d, *J*=8.0 Hz, 2H), 7.82 (d, *J*=8.0 Hz, 2H).

5-(*n*-Octyl)-4-tosyl-2-oxazoline (3f)

Yield: 78.3 %; mp 100.4-101.5 °C; ¹H NMR (CDCl₃): δ 0.88 (t, *J*=6.8 Hz, 3H), 1.21 (m, 12H), 1.64 (m, 2H), 2.42 (s, 3H), 4.73 (d, *J*=6.7 Hz, 1H), 5.03 (q, *J*=6.7 Hz, 1H), 6.97 (s, 1H), 7.34 (d, *J*=8.0 Hz, 2H), 7.80 (d, *J*=8.0 Hz, 2H).

5-(*n*-Nonyl)-4-tosyl-2-oxazoline (3g)

Yield: 97.1 %; mp 116.9-117.7 °C; ¹H NMR (CDCl₃): δ 0.88 (t, *J*=7.0 Hz, 3H), 1.25 (m, 14H), 1.68 (m, 2H), 2.42 (s, 3H), 4.75 (d, *J*=6.8 Hz, 1H), 5.04 (q, *J*=6.8 Hz, 1H), 6.95 (s, 1H), 7.35 (d, *J*=8.0 Hz, 2H), 7.82 (d, *J*=8.0 Hz, 2H).

5-(*n*-Decyl)-4-tosyl-2-oxazoline (3h)

Yield: 87.8 %; mp 105.8-106.4 °C; ¹H NMR (CDCl₃): δ 0.88 (t, *J*=7.0 Hz, 3H), 1.22 (m, 16H), 1.65 (m, 2H), 2.43 (s, 3H), 4.72 (d, *J*=6.7 Hz, 1H), 5.03 (q, *J*=6.7 Hz, 1H), 6.98 (s, 1H), 7.37 (d, *J*=8.0 Hz, 2H), 7.82 (d, *J*=8.0 Hz, 2H).

5-(Benzyl)-4-tosyl-2-oxazoline (4a)

Yield: 95.7 %; mp 93.8-96.7 °C; ¹H NMR (CDCl₃): δ 2.40 (s, 3H), 3.00 (m, 2H), 4.82 (d, *J*=6.7 Hz, 1H), 5.30 (q, *J*=6.7 Hz, 1H), 6.91 (s, 1H), 7.11-7.38 (m, 7H), 7.77 (d, *J*=8.0 Hz, 2H).

5-(2-Phenyl-ethyl)-4-tosyl-2-oxazoline (4b)

Yield: 75.8 %; mp 87.2-88.2 °C; ¹H NMR (CDCl₃): δ 1.99 (m, 2H), 2.44 (s, 3H), 2.78 (m, 2H), 4.79 (d, *J*=6.7 Hz, 1H), 5.07 (q, *J*=6.7 Hz, 1H), 7.00 (s, 1H), 7.01-7.40 (m, 7H), 7.78 (d, *J*=8.2 Hz, 2H).

5-(3-Phenyl-propyl)-4-tosyl-2-oxazoline (4c)

Yield: 68.7 %; mp 113.8-115.4 °C; ¹H NMR (CDCl₃): δ 1.73 (m, 4H), 2.44 (s, 3H), 2.65 (t, *J*=7.0 Hz, 2H), 4.71 (d, *J*=6.6 Hz, 1H), 5.07 (q, *J*=6.6 Hz, 1H), 6.95 (s, 1H), 7.09-7.32 (m, 5H), 7.35 (d, *J*=8.0 Hz, 2H), 7.78 (d, 8.0 Hz, 2H).

5-(4-Phenyl-butyl)-4-tosyl-2-oxazoline (4d)

Yield: 65.0 %; unstable product; ¹H NMR (CDCl₃): δ 1.48 (m, 2H), 1.66 (m, 4H), 2.42 (s, 3H), 2.62 (t, *J*=7.5 Hz, 2H), 4.73 (d, *J*=4.0 Hz, 1H), 5.03 (q, *J*=6.5 Hz, 1H), 6.94 (s, 1H), 7.07-7.44 (m, 7H), 7.79 (d, *J*=8.5 Hz, 2H).

5-(5-Phenyl-pentyl)-4-tosyl-2-oxazoline (4e)

Yield: 85.6 %; mp 104.2-105.4 °C; ¹H NMR (CDCl₃): δ 1.40 (m, 4H), 1.65 (m, 4H), 2.43 (s, 3H), 2.60 (t, J=7.6 Hz, 2H), 4.72 (d, J=6.5 Hz, 1H), 5.05 (q, J=6.5 Hz, 1H), 6.94 (s, 1H), 7.08-7.30 (m, 5H), 7.34 (d, J=8.0 Hz, 2H), 7.80 (d, J=8.0 Hz, 2H).

5-(6-Phenyl-hexyl)-4-tosyl-2-oxazoline (4f)

Yield: 54%; product unstable; ¹H NMR (CDCl₃): δ 1.29 (m, 6H), 1.54 (m, 4H), 2.34 (s, 3H), 2.53 (t, J=7.6 Hz, 2H), 4.69 (d, J=6.8 Hz, 1H), 5.06 (q, J=6.8 Hz, 1H), 6.90 (s, 1H), 6.99-7.19 (m, 5H), 7.29 (d, J=8.0 Hz, 2H), 7.74 (d, J=8.0 Hz, 2H).

5-(7-Phenyl-heptyl)-4-tosyl-2-oxazoline (4g)

Yield: 88.0 %; mp 101.4-102.2 °C; ¹H NMR (CDCl₃): δ 1.30 (m, 8H), 1.61 (m, 4H), 2.45 (s, 3H), 2.60 (t, J=8.2 Hz, 2H), 4.74 (d, J=6.8 Hz, 1H), 5.02 (q, J=6.8 Hz, 1H), 6.95 (s, 1H), 7.08-7.32 (m, 5H), 7.36 (d, J=8.0 Hz, 2H), 7.80 (d, J=8.0 Hz, 2H).

5-(8-Phenyl-octyl)-4-tosyl-2-oxazoline (4h)

Yield: 66.4 %; mp 98.4-99.3 °C; ¹H NMR (CDCl₃): δ 1.22 (m, 10H), 1.57 (m, 4H), 2.38 (s, 3H), 2.53 (t, J=7.3 Hz, 2H), 4.68 (d, J=6.6 Hz, 1H), 4.98 (q, J=6.6 Hz, 1H), 6.89 (s, 1H), 7.04-7.27 (m, 5H), 7.30 (d, J=8.0 Hz, 2H), 7.74 (d, J=8.0 Hz, 2H).

5-(9-Phenyl-nonyl)-4-tosyl-2-oxazoline (4i)

Yield: 69.3%; mp 94.0-95.1 °C; ¹H NMR (CDCl₃): δ 1.28 (m, 12H), 1.63 (m, 4H), 2.43 (s, 3H), 2.58 (t, J=7.4 Hz, 2H), 4.74 (d, J=6.6 Hz, 1H), 5.04 (q, J=6.6 Hz, 1H), 6.95 (s, 1H), 7.08-7.32 (m, 5H), 7.36 (d, J=8.0 Hz, 2H), 7.81 (d, J=8.0 Hz, 2H).

General Procedure for the preparation of 4-(*n*-alkyl)-1*H*-imidazoles and 4-(ω-phenylalkyl)-1*H*-imidazoles.

In a stainless steel bomb, a solution of the corresponding oxazoline (15.0 mmol) and a saturated solution of ammonia in abs. ethanol (120 mL) was heated at 120 °C for 25 h. The pressure increased to about 12 atm. After cooling, the solvent was removed under reduced pressure. The dark, oily residue was dissolved in ethyl acetate/dichloromethane (150 mL, 4/1, v/v) and washed with saturated aqueous sodium chloride (5x50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The isolated oil was purified on a silicagel column with CH₂Cl₂/MeOH (5/1, v/v) as eluent (for all products: 0.6<R_f<0.7). The product was crystallised as hydrogen oxalate from acetone/CH₃CN.

4-(*n*-Propyl)-imidazole (hydrogen oxalate) VUF 5522 (5a)

Yield: 44.7 %; mp 153.5-153.9 °C; ^1H NMR (DMSO- d_6): δ 0.83 (t, $J=7.3$ Hz, 3H), 1.57 (m, 2H), 2.58 (t, $J=6.7$ Hz, 2H), 7.32 (s, 1H), 8.80 (s, 1H), 13.24 (bs, 2.9 H). ^{13}C NMR (DMSO- d_6): δ 13.046, 21.158, 25.613, 115.353, 133.142, 135.349, 163.786.

Anal. calc. for $\text{C}_6\text{H}_{10}\text{N}_2 \cdot 1.0 \text{ C}_2\text{H}_2\text{O}_4$: C, 47.62; H, 5.97; N, 13.75. Found: C, 47.64; H, 5.69; N, 13.78.

4-(*n*-Butyl)-imidazole (hydrogen oxalate) VUF 5523 (5b)

Yield: 63.1 %; mp 155.6 °C; ^1H NMR (DMSO- d_6): δ 0.89 (t, $J=7.3$ Hz, 3H), 1.30 (m, 2H), 1.58 (m, 2H), 2.62 (t, $J=7.0$ Hz, 2H), 7.28 (s, 1H), 8.69 (s, 1H), 12.23 (bs, 2H). ^{13}C NMR (DMSO- d_6): δ 13.318, 21.226, 23.502, 29.930, 115.402, 133.373, 133.473, 164.012.

Anal. calc. for $\text{C}_7\text{H}_{12}\text{N}_2 \cdot 1.0 \text{ C}_2\text{H}_2\text{O}_4$: C, 49.97; H, 6.50; N, 12.81. Found: C, 50.04; H, 6.66; N, 12.80.

4-(*n*-Pentyl)-imidazole (hydrogen oxalate) VUF 5524 (5c)

Yield: 52.8 %; mp 179.8-180.4 °C, ^1H NMR (DMSO- d_6): δ 0.83 (t, $J=7.5$ Hz, 3H), 1.28 (m, 4H), 1.59 (m, 2H), 2.58 (t, $J=7.6$ Hz, 2H), 7.21 (s, 1H), 8.55 (s, 1H), 10.22 (bs, 1.6H). ^{13}C NMR (DMSO- d_6): δ 13.614, 21.511, 24.074, 27.653, 30.356, 115.674, 133.464, 133.776, 164.216. Anal. calc. for $\text{C}_8\text{H}_{14}\text{N}_2 \cdot 0.8 \text{ C}_2\text{H}_2\text{O}_4$: C, 54.84; H, 7.48; N, 13.32. Found: C, 54.80; H, 7.52; N, 13.22.

4-(*n*-Hexyl)-imidazole (hydrogen oxalate) VUF 5466 (5d)

Yield: 39.8 %; ^1H NMR (DMSO- d_6): δ 0.86 (t, $J=6.8$ Hz, 3H), 1.27 (m, 6H), 1.58 (m, 2H), 2.60 (t, $J=7.7$ Hz, 2H), 7.28 (s, 1H), 8.70 (s, 1H), 11.14 (bs, 1H). ^{13}C NMR (DMSO- d_6): δ 13.683, 21.737, 23.855, 27.779, 27.818, 30.616, 115.428, 133.385, 133.517, 163.777. Anal. calc. for $\text{C}_9\text{H}_{16}\text{N}_2 \cdot 1.1 \text{ C}_2\text{H}_2\text{O}_4$: C, 53.54; H, 7.30; N, 11.15. Found: C, 53.62; H, 7.37; N, 11.21.

4-(*n*-Heptyl)-imidazole (hydrogen oxalate) VUF 5526 (5e)

Yield: 55.4 %; mp 186.1-186.6 °C; ^1H NMR (DMSO- d_6): δ 0.84 (t, $J=6.7$ Hz, 3H), 1.25 (m, 8H), 1.58 (m, 2H), 2.60 (t, $J=8.0$ Hz, 2H), 7.26 (s, 1H), 8.58 (s, 1H). ^{13}C NMR (DMSO- d_6): δ 13.713, 21.823, 24.069, 27.954 (2x), 28.109, 30.917, 115.625, 133.454, 133.712, 163.914. Anal. calc. for $\text{C}_{10}\text{H}_{18}\text{N}_2 \cdot 0.8 \text{ C}_2\text{H}_2\text{O}_4$: C, 58.47; H, 8.29; N, 11.76. Found: C, 58.64; H, 8.38; N, 11.71.

4-(*n*-Octyl)-imidazole (hydrogen oxalate) VUF 5467 (5f)

Yield: 47.1 %; mp 190.2-190.7 °C; ¹H NMR (DMSO-d₆): δ 0.85 (t, J=7.0 Hz, 3H), 1.23(bs, 10H), 1.58 (m, 2H), 2.57 (t, J=6.8 Hz, 2H), 7.18 (s, 1H), 8.48 (s, 1H).

¹³C NMR (DMSO-d₆): δ 13.73, 21.85, 24.32, 28.07 (2x), 28.37, 28.42, 31.01, 115.88, 133.56, 133.96, 164.04. Anal. calc. for C₁₁H₂₀N₂·0.8 C₂H₂O₄: C, 59.98; H, 8.63; N, 11.10. Found: C, 60.12; H, 8.69; N, 11.13.

4-(*n*-Nonyl)-imidazole (hydrogen oxalate) VUF 5528 (5g)

Yield: 47.2 %; mp 191.0 °C; ¹H NMR (DMSO-d₆): δ 0.87 (t, J=6.8 Hz, 3H), 1.23 (bs, 12H), 1.58 (m, 2H), 2.58 (t, J=6.9 Hz, 2H), 7.32 (s, 1H), 8.78 (s, 1H). ¹³C NMR (DMSO-d₆): δ 13.723, 21.852, 23.758, 27.803, 28.091 (2x), 28.417, 28.632, 31.029, 115.337, 133.347, 133.430, 163.378. Anal. calc. for C₆H₁₀N₂·1.0 C₂H₂O₄: C, 60.20; H, 8.71; N, 10.17. Found: C, 60.52; H, 8.80; N, 10.15.

4-(*n*-Decyl)-imidazole (hydrogen oxalate) VUF 5529 (5h)

Yield: 68.6 %; mp 193.7-194.2 °C; ¹H NMR (DMSO-d₆): δ 0.85 (bs, 3H), 1.20 (bs, 14H), 1.58 (m, 2H), 2.59 (m, 2H), 7.24 (s, 1H), 8.66 (s, 1H). ¹³C NMR (400 MHz; 80 °C; DMSO-d₆): δ 13.425, 21.641, 24.400, 28.0182, 28.0972, 28.2474, 28.2991, 28.5372, 28.5599, 30.893, 115.796, 133.358, 134.182, 163.379. Anal. calc. for C₁₃H₂₄N₂·0.8 C₂H₂O₄: C, 62.55; H, 9.20; N, 9.99. Found: C, 62.59; H, 9.30; N, 10.03.

4-(benzyl)-imidazole (hydrogen oxalate) VUF 5511 (6a)

Yield: 31.4 % mp 172.7-175.4 °C; ¹H NMR (DMSO-d₆): δ 3.98 (s, 2H), 7.20 (s, 1H), 7.25 (m, 5H), 8.50 (s, 1H), 9.73 (s, 1H). ¹³C NMR (DMSO-d₆): δ 30.407, 116.341, 126.228, 128.252, 133.273, 134.019, 138.297, 164.572. Anal. calc. for C₁₀H₁₀N₂·0.75 C₂H₂O₄: C, 61.19; H, 5.14; N, 12.41. Found: C, 61.08; H, 4.69; N, 12.28.

4-(2-Phenylethyl)-imidazole (hydrogen oxalate) VUF 5512 (6b)

Yield: 55.9 %; mp 183.0-183.4 °C; ¹H NMR (DMSO-d₆): δ 2.91 (m, 4H), 7.13 (s, 1H), 7.14-7.33 (m, 5H), 8.52 (s, 1H), 10.74 (s, 2.2H). ¹³C-NMR (DMSO-d₆): δ 26.196, 33.918, 115.825, 125.818, 128.033, 128.83, 133.261, 133.541, 140.447, 164.548. Anal. calc. for C₁₁H₁₂N₂·0.8 C₂H₂O₄: C, 61.96; H, 5.61; N, 11.47. Found: C, 62.03; H, 5.81; N, 11.42.

4-(3-Phenyl-propyl)-imidazole (hydrogen oxalate) VUF 5513 (6c)

Yield: 74.4 %; mp 186.1-187.9 °C; ¹H NMR (DMSO-d₆): δ 1.91 (m, 2H), 2.62 (m, 4H), 7.04-7.38 (m, 5H), 7.41 (s, 1H), 8.78 (s, 1H), 9.45 (bs, 2.8H). ¹³C-NMR (DMSO-d₆): δ

23.372, 29.315, 34.013, 115.262, 125.479, 127.952, 132.920, 133.262, 141.009, 163.541. Anal. calc. for $C_{12}H_{14}N_2 \cdot 1.0 C_2H_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.97; H, 5.81; N, 10.08.

4-(4-Phenyl-butyl)-imidazole (hydrogen oxalate) VUF 5514 (6d)

Yield: 60.9 %; mp 178.9-180.6 °C; 1H NMR (DMSO- d_6): δ 1.59 (m, 4H), 2.61 (m, 4H), 6.81 (bs, 1H), 7.09-7.36 (m, 6H), 8.51 (s, 1H). ^{13}C NMR (DMSO- d_6): δ 23.985, 27.699, 30.117, 34.501, 115.707, 125.432, 128.002, 133.489, 133.694, 141.775, 164.346.

Anal. calc. for $C_{13}H_{16}N_2 \cdot 0.75 C_2H_2O_4$: C, 65.03; H, 6.59; N, 10.46. Found: C, 65.02; H, 6.64; N, 10.37.

4-(5-Phenyl-pentyl)-imidazole (hydrogen oxalate) VUF 5515 (6e)

Yield: 86.2 %; mp 167.1-168.2 °C; 1H NMR (DMSO- d_6): δ 1.32 (m, 2H), 1.61 (m, 4H), 2.59 (m, 4H), 7.07-7.34 (m, 6H), 7.60 (bs, 1H), 8.73 (s, 1H). ^{13}C NMR (DMSO- d_6): δ 23.738, 27.637 (2x), 30.308, 34.684, 115.390, 125.386, 127.978, 128.022, 133.299, 133.405, 141.908, 163.709. Anal. calc. for $C_{14}H_{18}N_2 \cdot 1.0 C_2H_2O_4$: C, 63.14; H, 6.62; N, 9.20. Found: C, 63.12; H, 6.62; N, 9.15.

4-(6-Phenyl-hexyl)-imidazole (hydrogen oxalate) VUF 5516 (6f)

Yield: 40.9 %; mp 184.1-185.6 °C; 1H NMR (DMSO- d_6): δ 1.30 (m, 4H), 1.56 (m, 4H), 2.68 (m, 4H), 7.08-7.37 (m, 6H), 8.53 (s, 1H), 8.59 (bs, 1H). ^{13}C NMR (DMSO- d_6): δ 24.135, 27.920, 27.980, 28.039, 30.632, 34.849, 115.683, 125.349, 127.967, 128.007, 133.424, 133.750, 142.002, 164.311. Anal. calc. for $C_{15}H_{20}N_2 \cdot 0.75 C_2H_2O_4$: C, 66.98; H, 7.32; N, 9.47. Found: C, 66.93; H, 7.33; N, 9.44.

4-(7-Phenyl-heptyl)-imidazole (hydrogen oxalate) VUF 5517 (6g)

Yield: 83.0 %; mp 164.9-166.3 °C; 1H NMR (DMSO- d_6): δ 1.28 (m, 6H), 1.56 (m, 4H), 2.58 (m, 4H), 7.08-7.34 (m, 6H), 8.72 (s, 1H), 9.77 (bs, 1H). ^{13}C NMR (DMSO- d_6): δ 23.683, 27.762, 28.031, 28.193, 28.282, 30.732, 34.881, 115.270, 125.343, 127.971, 128.002, 133.285, 133.355, 142.039, 163.607. Anal. calc. for $C_{16}H_{22}N_2 \cdot 1.0 C_2H_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: C, 65.12; H, 7.22; N, 8.44.

4-(8-Phenyl-octyl)-imidazole (hydrogen oxalate) VUF 5518 (6h)

Yield: 96.4 %; mp 139.9-147.1 °C; 1H NMR (DMSO- d_6): δ 1.24 (m, 8H), 1.56 (m, 4H), 2.58 (m, 4H), 6.98 (bs, 1.6H), 7.03-7.40 (m, 6H), 8.79 (s, 1H). ^{13}C NMR (DMSO- d_6): δ 23.651, 27.745, 28.055, 28.362, 28.482, 30.763, 34.902, 115.226, 125.332, 127.963,

127.993, 133.259, 133.319, 142.047, 163.332. Anal. calc. for $C_{17}H_{24}N_2 \cdot 1.0 C_2H_2O_4$: C, 65.88; H, 7.56; N, 8.09. Found: C, 65.78; H, 7.61; N, 8.09.

4-(9-Phenyl-nonyl)-imidazole (hydrogen oxalate) VUF 5519 (6i)

Yield: 87.4 %; mp 157.2-159.5 °C; 1H NMR (DMSO- d_6): δ 1.25 (m, 10H), 1.56 (m, 4H), 2.58 (m, 4H), 7.05-7.33 (m, 6H), 8.00 (bs, 1H), 8.72 (s, 1H). ^{13}C NMR (DMSO- d_6): δ 23.738, 27.787, 28.079, 28.395, 28.582, 28.621, 30.780, 34.910, 115.289, 125.339, 127.965, 128.005, 133.284, 133.384, 142.058, 163.497. Anal. calc. for $C_{18}H_{26}N_2 \cdot 1.0 C_2H_2O_4$: C, 66.64; H, 7.83; N, 7.77. Found: C, 66.65; H, 7.81; N, 7.93.

Pharmacology

The histamine H_3 activity of the compounds was determined on an in vitro assay, on the basis of the inhibitory effect of histamine H_3 agonists on electrically evoked twitches (induced by endogenous acetylcholine release) of guinea pig jejunum preparations.¹⁹

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Chapter 8

Development of a qualitative pharmacophore model for histamine H₃ receptor antagonists, using the newly developed molecular modelling program SLATE

Abstract

New molecular modelling tools were developed to construct a pharmacophore model for histamine H₃ receptor antagonists. The program SLATE superposes ligands assuming optimum hydrogen-bond geometry. One or two ligands are allowed to flex in the procedure, thereby enabling the determination of the bioactive conformation of flexible H₃ antagonists. In the derived model, four hydrogen-bonding site points and two hydrophobic pockets available for binding antagonists are revealed. The model results in a better understanding of the structure activity relationships of H₃ antagonists. To validate the model, a series of new antagonists was synthesised. The compounds were designed to interact with all four hydrogen-bonding site points and the two hydrophobic pockets simultaneously. These ligands have the anticipated high H₃ receptor affinity, thereby illustrating how the model can be used in the design of new classes of H₃ antagonists.

Introduction

Histamine mediates its actions via the stimulation of three distinct receptor subtypes, H₁, H₂ and H₃.¹ Selective antagonists of H₁ and H₂ receptors have been very successful in the treatment of allergic reactions and gastric ulcers, respectively, but the therapeutic use of H₃ receptor-related drugs has yet to be established. However, since the discovery of the H₃ receptor in 1983 by Arrang and co-workers,² considerable progress has been made in understanding the role of the H₃ receptor in (patho)physiology. This receptor controls neuronal synthesis of histamine³ and in addition regulates the release of the neurotransmitter into the synaptic cleft.⁴ Furthermore, it acts as a heteroreceptor at *e.g.*, serotonergic, noradrenergic, cholinergic, dopaminergic and peptidergic neurons.⁵ H₃ receptors have been identified in peripheral tissues⁶, but the highest densities have been found in distinct areas of the central nervous system, especially in the areas that are associated with cognition.⁷ Pharmacological studies have provided clear indications for the clinical use of selective H₃ ligands and centrally active H₃ antagonists are considered potential therapeutic agents to treat neurological disorders like obesity, epilepsy, sleeping disorders, and cognition and memory deficits.⁸ Molecular modelling studies can give a fresh impetus to the design of new ligands. In chapter 4, a pharmacophore model for

histamine H₃ antagonists is presented.⁹ The interaction of selected H₃ ligands with an aspartate residue of the receptor that is available for ligand binding was investigated. As two distinct lipophilic pockets available for ligand binding were revealed, the derived model explains the differences in structure activity relationship (SAR) observed for the lipophilic tails of different classes of antagonists. However, detailed information about the relative location and shape of the distinct lipophilic pockets could not be obtained as the computational costs of the methods applied (a density functional approach using parallel supercomputers) prompted the truncation of the lipophilic tails of the antagonists to more manageable methyl groups. Furthermore, several classes of H₃ antagonists had to be omitted in the preliminary model as they lack a basic moiety that interacts with the aspartate residue.

Molecular similarity studies of the H₃ antagonists can be deceptive as several studies^{9,10} have indicated that different classes of antagonists interact with different sets of (receptor) site points, *i.e.*, indicating *partial* similarity.^{11,12} Furthermore, modelling studies are seriously hampered by the flexibility of the H₃ ligands. Synthetic approaches to solve this latter problem have not led to the necessary highly active, conformationally restrained compounds which can serve as templates in subsequent, more quantitative modelling studies (chapter 5 and chapter 6).

To overcome the described problems, a new molecular modelling tool has been developed. The program SLATE superposes two flexible ligands assuming optimum hydrogen-bonding geometry. During the optimisation, the ligands are represented by specific points used for superposition, *i.e.*, ligand hydrogen-bonding acceptor atoms, receptor hydrogen-bonding acceptor atoms (given by projecting ligand donor hydrogen atoms towards the optimum hydrogen-bonding position of the site) and aromatic rings. For each ligand, the 3D arrangement of the points used for superposition (the "pharmacophore") is expressed as a distance matrix (Figure 1). After every conformational change the difference distance matrix is calculated by subtraction of the distance matrices. The objective function that is minimised by SLATE is the sum of the difference distance matrix elements, *i.e.*, the score for the degree of similarity of the "pharmacophores" described by the two ligands. Simulated annealing is used for this minimisation,¹³ which enables the rapid identification of good solutions, ideally the global minimum. The procedure involves changing only the torsion angles and the relative ordering of corresponding points, and is rapid enough to allow one or both ligands to flex. The resulting, optimised conformations are superposed (by rotation and transformation, keeping the derived conformations fixed) using the MATFIT algorithm,¹⁴ a so-called rigid fit procedure. Details and validation of SLATE will be described elsewhere.¹⁵

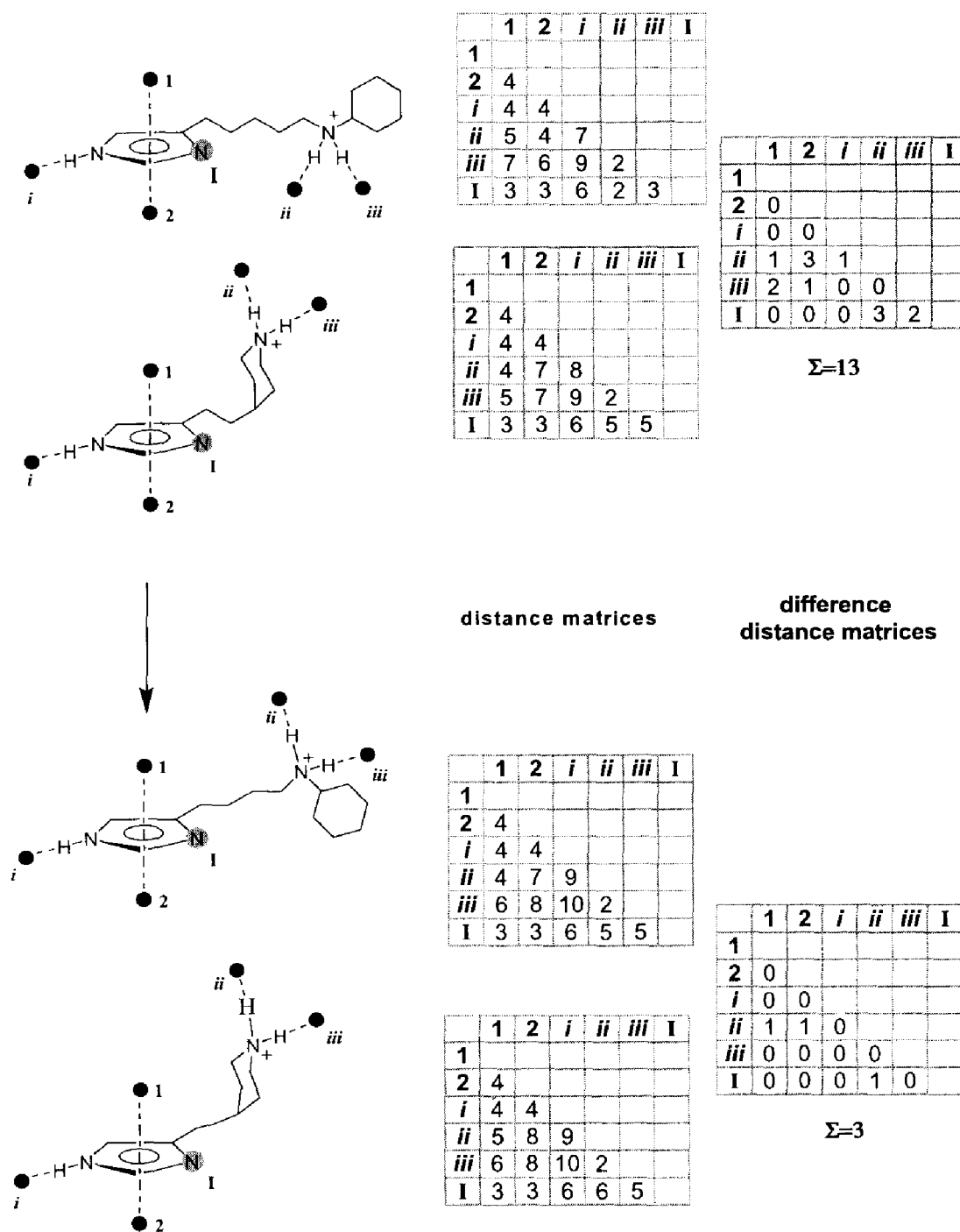


Figure 1. Schematic representation of the objective function calculated by the program SLATE. In this example, the points used for superposition are the ligand hydrogen-bonding acceptor atoms (**I**), the receptor hydrogen-bonding acceptor atoms (*i*, *ii* and *iii*) and the two points on either side of the vector passing through aromatic centroids perpendicular to aromatic rings (1 and 2). The distances between these points are expressed in the distance matrix and a difference distance matrix is calculated by subtraction of distance matrices. The objective function in SLATE is the sum of the difference distance matrix elements. See text for details.

The ligands

Different classes of H₃ antagonists are known; for a thorough review the reader is referred to chapter 1. One highly potent representative of several classes is shown in Figure 2. The most conserved feature of these H₃ ligands is the 4(5)-substituted imidazole ring that is essential for activity within these series. Whereas additional substituents on the imidazole ring lead to a dramatic reduction in activity, replacement of the imidazole by other functional groups results in compounds with no H₃ activity at all. This crucial role of the imidazole ring in most classes of H₃ antagonists indicates that at least this part of the ligands binds to the same receptor site and that this interaction is compulsory. It has to be noted that, very recently, new classes of H₃ antagonists have been described that lack an imidazole ring.¹⁶ It has yet to be established whether these compounds interact at the same binding site of the H₃ receptor as the imidazole-containing ligands.

Several classes of antagonists shown in Figure 2 have miscellaneous functional groups that contain a basic nitrogen atom (1, 2, 3, 7, 8, 9, 10, 11, 13). These functional groups are attached to the imidazole ring via linkers of various length. These basic pharmacophoric elements can interact with a hydrogen-bonding acceptor site point.^{9,10} Attachment of a lipophilic residue ("tail") to the basic moiety can significantly influence the H₃ activity. For many classes of antagonists, cycloalkyl groups or (halogenated) benzyl groups lead to the most potent compounds within the given series.

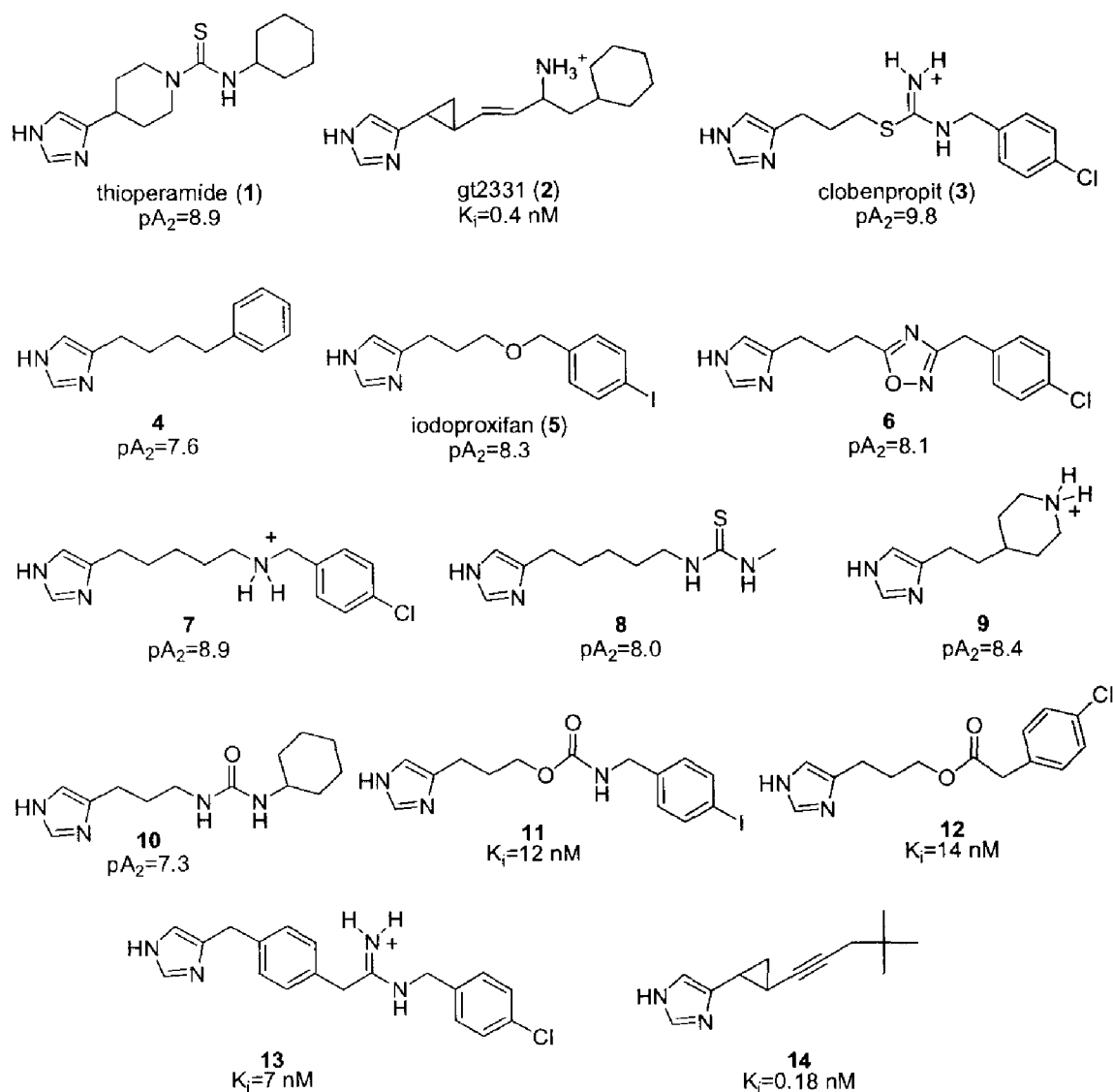


Figure 2. The H₃ antagonists studied. The biological data are determined by evaluation of the influence of the compound on electrically-evoked, cholinergic contractions of guinea pig intestine preparations (pA_2) or by evaluation of the influence of the compound on K⁺-stimulated [³H]-histamine release on rat cortex (pK_i).³⁷

Some classes of antagonists lack a basic nitrogen atom in their imidazole side chain (4, 5, 6, 12, 14). Such compounds bind at the imidazole-binding site and a lipophilic pocket, obviously without interacting with the aforementioned hydrogen-bonding site point. The available data suggest that these ligands have a similar SAR with respect to the terminal lipophilic moiety, *i.e.*, cycloalkyl or substituted benzyl groups leading to the most potent compounds. However, the SAR concerning the terminal lipophilic moiety throughout the different classes of H₃ antagonists is far from unambiguous and many authors have suggested different binding sites for H₃ antagonists. For example, Stark and co-workers

have proposed a different, unique binding mode for the isothiourea derivatives of **3**, as substitution of the isothiourea group by halogenated benzyl groups leads to far more potent compounds than substitution with cycloalkyl groups.¹⁷ Several authors have suggested an alternative binding mode for thioperamide (**1**)^{18,19,20,21} and its derivatives and Vollinga *et al.* described a class of antagonists (represented by **8**) lacking a significant influence of the type of lipophilic terminus on the activity.²² None of these findings have been rationalised firmly.

Methods

The molecular co-ordinates of the ligands were constructed using MacroModel.²³ The basic nitrogen atoms in the side chain of the ligands were protonated except the nitrogen atoms of the urea, thiourea and carbamate groups.^{24,25} Since, at present, no indication exists about the bioactive tautomeric form of the imidazole moiety,²⁶ the N^t-H tautomer was arbitrarily selected for all investigated compounds. Aromatic regions were included in the matching procedure to ensure a tight superposition of the imidazole rings. The torsion angles of all single bonds were affected by the optimisation procedure. Furthermore, the bonds of the isothiourea, thiourea and amidine moieties were allowed to flip (180°) during the optimisation to generate all possible configurations. For the structures containing a piperidine ring, all different ring conformers were generated and used as starting geometries. To this end, MacroModel²³ was used for molecular mechanics conformational analysis of the different possible ring structures (using the ring closure bond option). By rotating all bonds through 360° with increments of 10°, a large number of conformations was generated. These conformations were energy optimised using the Amber force field²⁷ with the program Batchmin 2.7²³ in order to obtain low energy conformations.

All trials using SLATE were run under the default annealing conditions.¹⁵ Null correspondences were introduced according to literature procedures.^{28,29} The so-called difference-distance matrix distance threshold parameter (see Mills and co-workers¹⁵ for more details) was set equal to the high accuracy of 0.0001, to force exact superposition of pharmacophore points where possible. Fifty trials for each pair of molecules were run and the trials in each set were ranked according to their steric similarity, calculated as the fraction of surface volume overlap by the program PLM.³⁰ For the superposed structures, the most likely positions of the complementary hydrogen-bonding atoms in the binding site were predicted using the program DOH.²⁹ Compounds **12** and **13** were fitted onto the pharmacophore using PSEUDO.³¹ This program superposes a flexible ligand on a template by maximising the overlap of the molecular skins. For each compound fifty trials were run

(using the default simulated annealing settings¹⁵). The fits with the best steric score were selected.

Construction of the pharmacophore

Initially, the lipophilic tails of the antagonists used to construct the pharmacophore, were truncated to methyl groups to facilitate steric evaluation of the fits and to circumvent the anticipated partial similarity problems that may accompany these substructures (*vide supra*). The approach of pairwise matching was applied, *i.e.*, looking for one unique conformation of a reference ligand that is found in superposition trials with different ligands. Thioperamide (**1**) was selected as reference structure. First, **1** (with the piperidine ring in the chair conformation) and GT2331 (**2**) were superposed by running fifty trials of SLATE, allowing both compounds to flex. The fit with the best steric score is shown in Figure 3. In total, six different conformations of **1**, called set I, were found to match different conformations of **2**. In a similar experiment, thioperamide (**1**) and clobenpropit (**3**) were superposed, allowing both ligands to flex. Many different conformations of the ligands within this set II were found. However, comparison of the conformations of thioperamide (**1**) in set I and set II revealed that only one conformation was found in both sets. Furthermore, this unique conformation of thioperamide was found in the superpositions that had the highest steric similarity score in both sets I (Figure 3) and II (not shown). Therefore, this conformation of thioperamide, and the corresponding conformation of **2** and **3**, were accepted as the bioactive conformations. A unique pharmacophore could only be found when the piperidine ring of **1** was in the chair conformation. No identical conformations of the reference ligand **1** in the different sets could be found when the piperidine ring was (fixed) in a different conformation.

As can be seen in Figure 3, the methyl groups (that substitute the lipophilic tails) of the antagonists **1** and **2** have a different position and orientation, indicating that this procedure finds two lipophilic pockets available for antagonist binding, thereby validating earlier findings.⁹ Figure 3 also illustrates that it is not necessary to force the basic nitrogen of the ligands to occupy the same position in space. A certain degree of positional freedom for these substructures is allowed to obtain an optimal position to interact with the complementary hydrogen-bonding site points, hence taking into account the directionality of the intermolecular hydrogen bond.

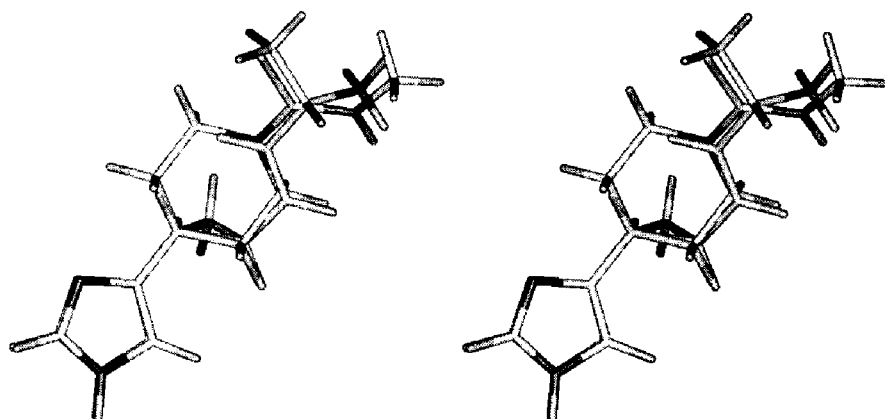


Figure 3. Construction of the pharmacophore by superimposing **1** and **2**, letting both compounds flex. The fit with the highest steric similarity is shown (stereoview).

The lipophilic tail of thioperamide (**1**), a cyclohexyl group, was added using the program MacroModel. This substructure was energy optimised using the Amber force field²⁷ while the positions of all other atoms of the template were frozen.

The relative position of the lipophilic tail of clobenpropit (**3**), a 4-Cl-benzyl group, was determined using SLATE. The results of the study described in chapter 7 were necessary for the determination of the position of this pocket. Again, the approach of pairwise matching was used. The complete flexible antagonists **4**, **5** and **6**, were matched with clobenpropit **3** that was fixed in the bioactive conformation (as described in a previous subsection) except for the lipophilic tail and the bonds of the isothiourea group, which were allowed to flip 180°. Comparing the three sets as described before revealed the unique relative position of the lipophilic tails.

Having derived the relative position and orientation of the imidazole moieties (and therefore hydrogen-bonding site points A and B), the basic groups in the imidazole side chain (interacting with hydrogen-bonding site point C) and the lipophilic tails of these ligands (see Figure 4). Subsequent ligands were fitted onto this pharmacophore. In all cases, the fit with the highest steric score was selected. This procedure revealed a fourth hydrogen-bonding site point (D). Obviously, this site point introduces another cause for partial similarity. It has to be noted that the position of site point D was confirmed by using an analogous approach as described before, *i.e.*, using SLATE to determine the bioactive conformations of two (different) ligands by allowing both structures to flex.

Results and discussion

The antagonistic binding site of the histamine H₃ receptor can be described by four hydrogen-bonding site points and two lipophilic pockets. The relative orientation of these features is shown in Figure 4 by superposition of ligands **1**, **3** and **9**, which also illustrates the steric requirements of the derived pharmacophore. The imidazole moiety of the ligands interacts with site points A and B. Basic nitrogen atoms of the imidazole side chain of the ligands can interact with site points C and/or D. Only clobenpropit (**3**) is able to interact with all four hydrogen-bonding site points simultaneously. This might explain the high potency of this antagonist. In addition, we recall that it has been suggested that clobenpropit and its derivatives have a different binding mode since cycloalkyl groups as lipophilic tails are less effective than substituted benzyl groups (*vide infra*). The interaction of the isothioureia group with two hydrogen-bonding acceptor site points might benefit significantly from electron-withdrawing groups, *e.g.*, halogenated benzyl groups. This electronic effect will be less prominent for the other classes of antagonists.

Pocket 1 is occupied by antagonists **2**, **3**, **4**, **5**, **6**, **7**, **10**, **11**, **12** and **13** (Figure 5). These antagonists have cycloalkyl- and (substituted-) benzyl- groups. The hydrophobic region is easily accessible and (as is apparent from the SAR) with the proper lipophilic tail, a high increase in affinity is obtained. The lipophilic tails of **1**, **8** and **14** interact with hydrophobic pocket 2 (Figure 5). The entrance to this pocket seems to be rather narrow, being enclosed by the two site points C and D. The thioureia moieties of ligands **1** and **8** are only partly overlapping, so the orientation, and hence the SAR, of the lipophilic tails are different.

Figure 4. Superposition of ligands **1**, **3** and **9** (stereoview) illustrate the position of the four hydrogen-bonding site points (A-D) and two lipophilic pockets (1 and 2) and gives an indication about the steric requirements of the derived pharmacophore. Carbon atoms are shown in green, oxygen atoms in red, nitrogen atoms in blue, sulphur atoms in yellow and hydrogen atoms in white. Acceptor site points are shown in magenta and donor site points in yellow.

Figure 5. Superposition of ligands 1-12 (stereoview). Hydrogen atoms attached to carbon atoms are omitted.

Figure 6. Detailed description of the shape of pocket 2 by superposing **1** (yellow), **8** (ball and stick), **15** and **16**. See text for details.

Figure 7. Superposition of **1** and **10**. Compound **1** occupies pocket 2, compound **10** occupies pocket 1. The thiourea moiety of **1** cannot adopt a *trans-cis* conformation as this would lead to intra-molecular clashes (note the vicinity of the cyclohexyl ring of **10** and the piperidine ring of **1**).

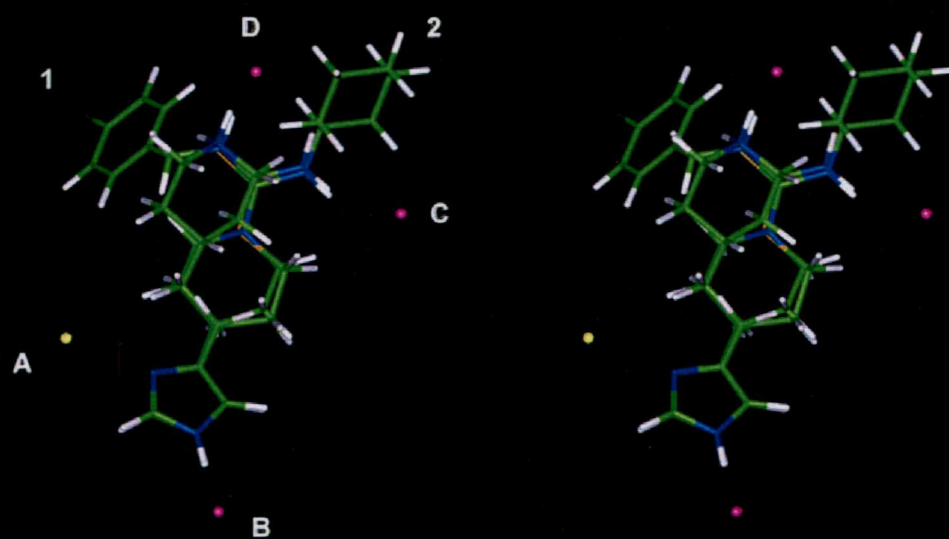


Fig. 4

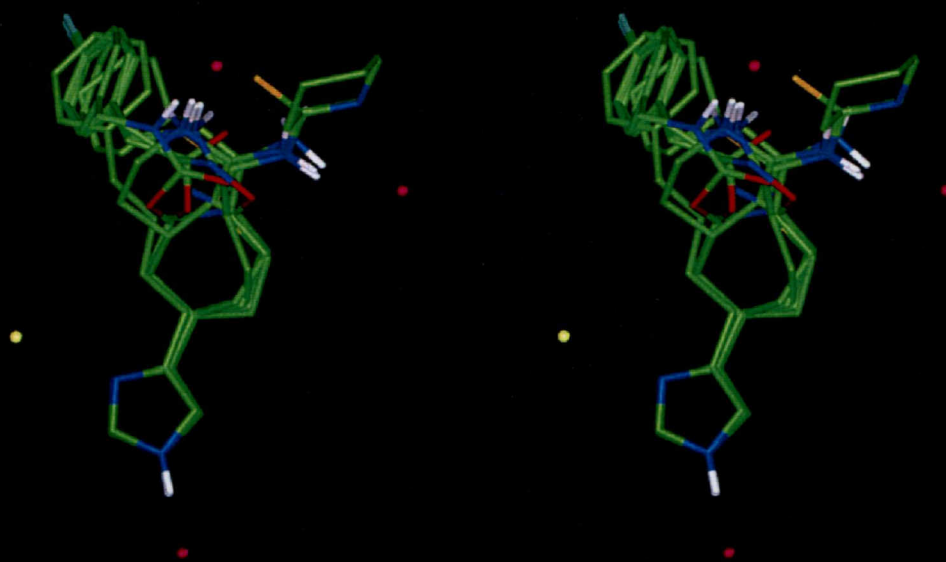


Fig. 5

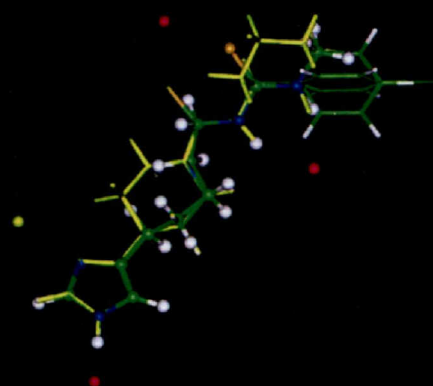


Fig. 6

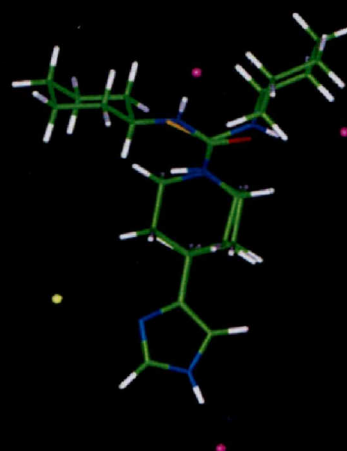
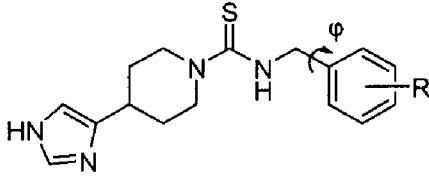


Fig. 7

Detailed information about the shape of pocket 2 was obtained by incorporation of the results of a QSAR study.¹⁸ It was reported that substitution of the cyclohexyl group of **1** by substituted benzyl groups leads to ligands with reduced activity. The position of the substituent on the benzyl group had a remarkable effect on the activity, with ortho-substituents being more favourable for H₃ antagonistic activity than para-substituents. In this QSAR study, using 15 thioperamide derivatives, it was revealed that the latter effect was caused by the unique character of the piperidine-1-carbothioic acid amide moiety. A correlation was found between the activity of the different analogues and the torsion angle ϕ of these compounds (illustrated in Table 1).

Table 1. Histamine H₃ antagonistic activity and calculated torsion angle ϕ of two selected thioperamide derivatives as published in a QSAR study by Windhorst and co-workers.¹⁸

			
Compound	R	pA ₂ (\pm sem)	ϕ
15	2-Cl	8.21 (0.09)	-168.5°
16	4-Cl	7.21 (0.08)	-122.2°

Incorporation of two of these conformations in the present model gives a rationale for this observation (Figure 6). The 2-Cl benzyl group of **15** uses the same plane in 3D space as the methyl group of **8** and the cyclohexyl group of **1**, but the 4-Cl benzyl group of **16** is twisted out of this plane. Therefore, the model indicates that the latter position is not available for antagonists.

The binding mode of thioperamide (**1**) can be illustrated by yet another peculiarity, noted by Leurs *et al.*²¹ These authors were puzzled by the finding that replacement of the thiourea group of thioperamide **1** by a urea group leads to a significant decrease in affinity, while the replacement of the thiourea group in its flexible analogue norburimamide by an urea group (leading to **10**) has no effect on the activity. It has to be noted that the activities were obtained using different pharmacological assays, making a straightforward comparison difficult. Nevertheless, the model indicates putative differences in binding modes of ligands **1** and **10**. The basic urea moiety of (**10**) can adopt a *trans-cis* configuration, interacting with site point D and orientating its lipophilic tail towards pocket 1 (Figure 7). However, thioperamide (**1**) can only interact with site point C and occupy pocket 2. The

thiourea moiety of this compound cannot adopt a *cis* configuration as this would cause intramolecular clashes between the piperidine ring and the cyclohexyl tail. The position of the sulphur atom of thioperamide (**1**) is therefore restricted to a position near hydrogen-bonding site point D. It can be speculated that the (negative) electrostatic potential, induced by the oxygen atom of the urea group, cannot be accommodated in this region.

Surprisingly, compound **13** could not be fitted into the pharmacophore using SLATE. Therefore, the program PSEUDO was applied. This program superposes a flexible ligand on a template by maximising the overlap of the molecular skins.³¹ Using PSEUDO under the default conditions,³¹ a conformation of ligand **13** was derived (Figure 8) in which the amidine moiety does not interact with the hydrogen-bonding acceptor site points C or D, explaining why SLATE did not find this conformation. No data for the role of the amidine group are available, but the theoretical considerations presented here indicate that this group is not involved in hydrogen-bonding interaction with the receptor. The program PSEUDO was also used to fit compound **14**. No structural variations of the lipophilic tail of this antagonist (*i.e.*, the *tert*-butylgroup) have been reported. Thus, no experimental data is available that could indicate whether the compound interacts with pocket 1 or pocket 2. The program PSEUDO suggests that the *tert*-butylgroup of **14** occupies pocket 2 (Figure 9). One of the methyl groups of this moiety is positioned just above the centre of the cyclohexyl ring of thioperamide. This is in perfect agreement with recently published data in which it was shown that replacement of the cyclohexyl ring of thioperamide (**1**) by an adamantyl group results in a slightly more potent H₃ antagonist.³² The chiral cyclopropyl units of **2** and **14** occupy exactly the same position in 3D space as might be expected when considering the stereospecificity of the H₃ receptor (for details see chapter 1).

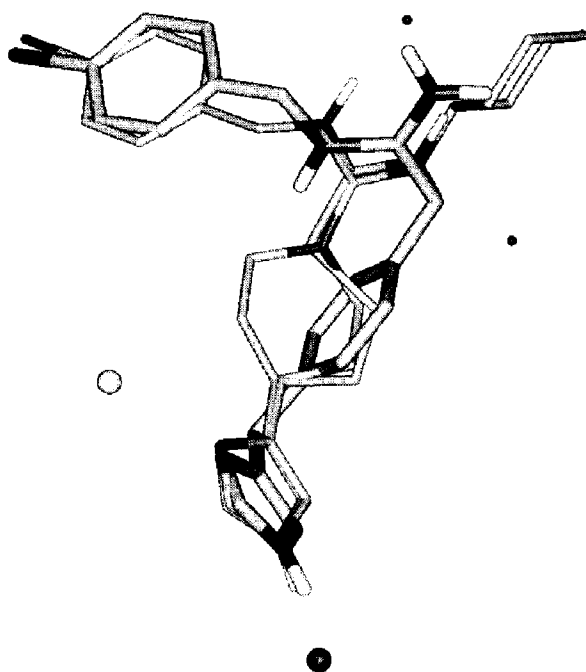


Figure 8. Superposition of **1**, **7** and **13** (the latter in bold). Compound **13** does not interact with site point C or D. Hydrogen atoms attached to carbon atoms are omitted.

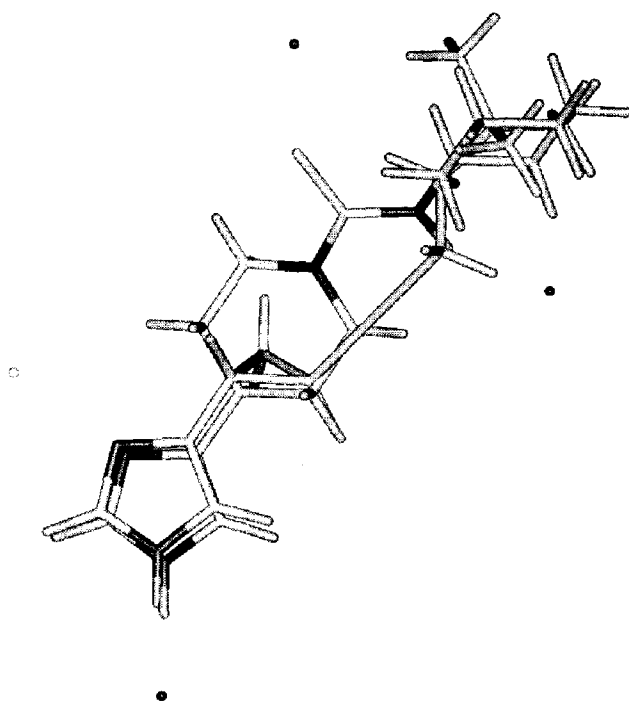


Figure 9. Superposition of **1** and **14**.

To validate the presence of two hydrophobic pockets available for H₃ antagonist binding, a small series of compounds was synthesised and the affinity of the ligands for the H₃ receptor was determined. Clobenpropit (**3**) is an ideal lead compound, as a (substitutable) hydrogen atom of the isothioureia moiety is pointing directly towards the second hydrophobic pocket. On the basis of the pharmacophore model, the receptor can accommodate a cyclohexyl group in this position, as is illustrated in Figure 10 by the superposition of clobenpropit (**3**) and thioperamide (**1**). In addition to the interaction with two hydrophobic pockets, the designed compounds are predicted to have interaction with all four hydrogen-bonding site points.

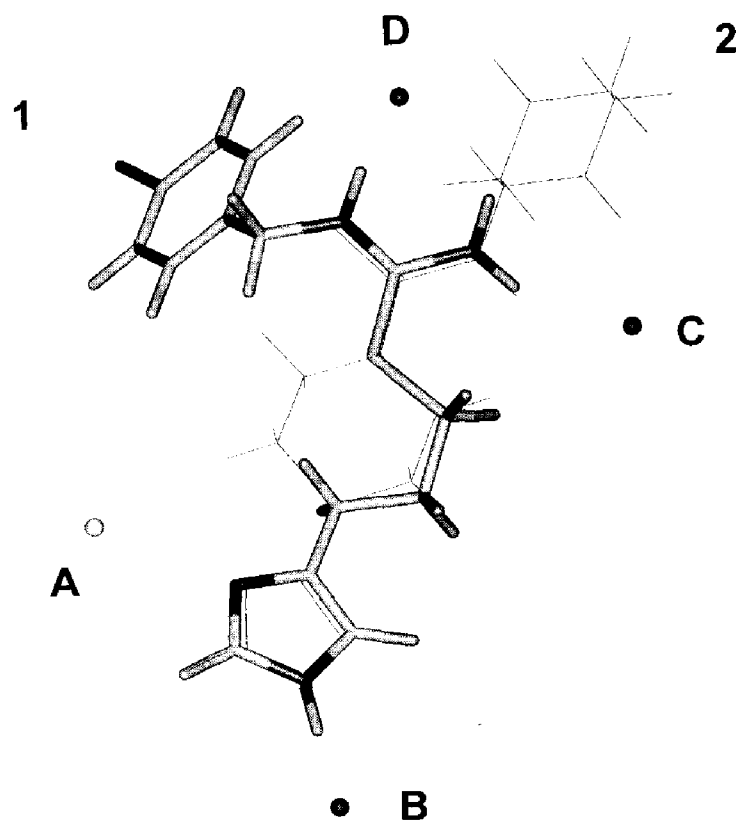
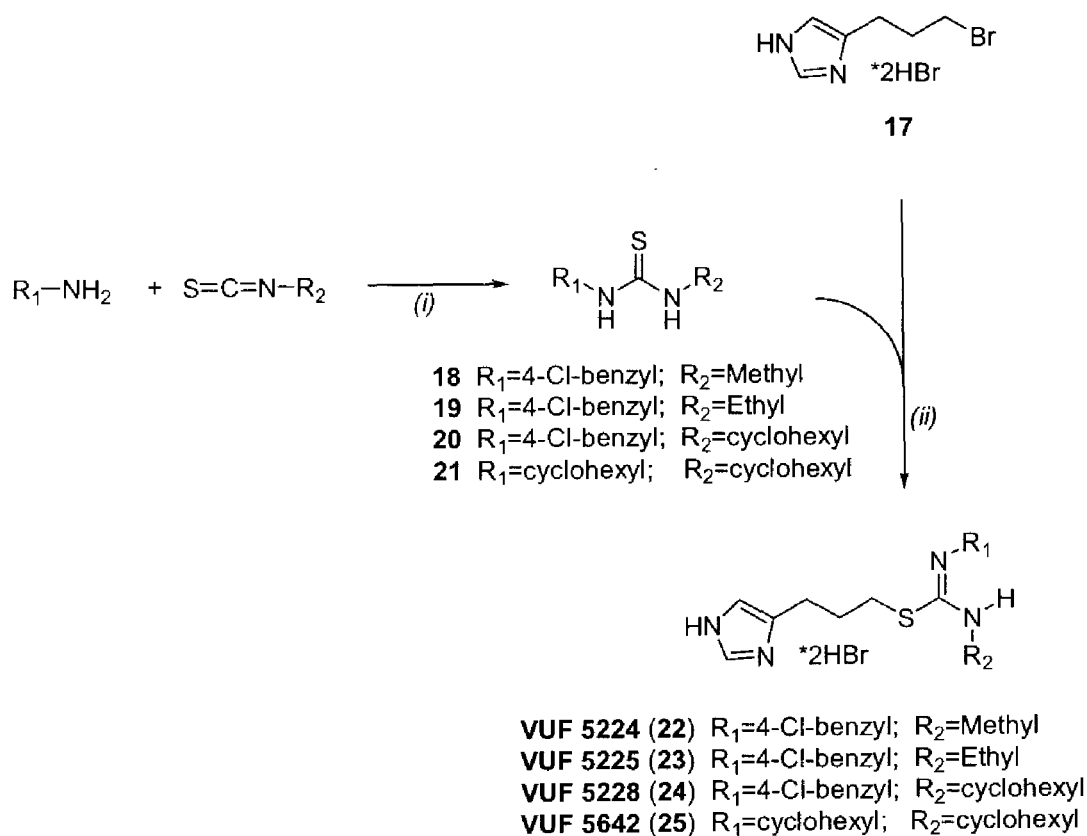


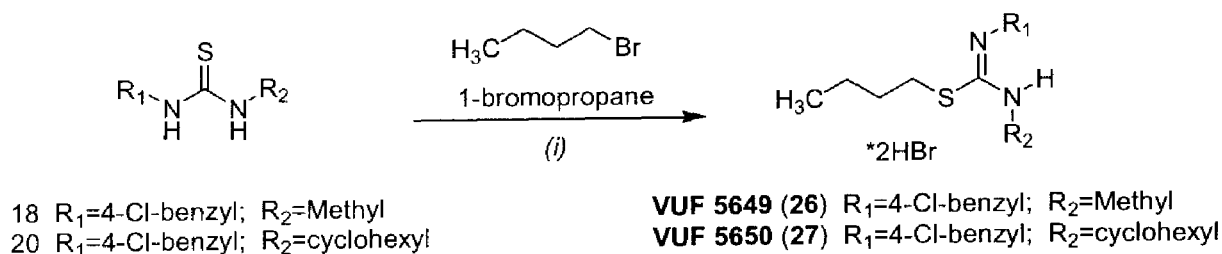
Figure 10. Superposition of thioperamide (**1**) (in feint) and clobenpropit (**3**).

The ligands **22-25** were synthesised according to scheme 1. Intermediate 4-(3-bromopropyl)-1*H*-imidazole hydrobromide (**17**), which was synthesised according to literature procedure, was treated with thiourea compounds (**18-21**) to yield the target ligands (**22-25**). The thiourea compounds (**18-21**) were prepared from commercially available isothiocyanates and the corresponding amines.



Scheme 1. (i) DCM; (ii) ethanol, Δ .

To investigate the necessity of the imidazole moiety, the two non-imidazole compounds **26** and **27** were synthesised, via an analogous route (scheme 2).

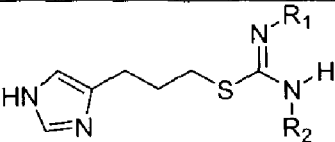


Scheme 2. (i) ethanol, HBr, Δ .

Pharmacology

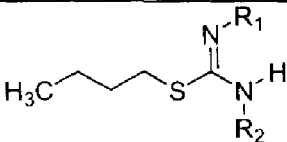
The affinity for the histamine H₃ receptor was determined by displacement of the radioligand [³H] N^α-methylhistamine using membrane homogenates of rat cerebral cortex.

Table 2. Histamine H₃ receptor binding affinity of the selected compounds.

				
compound	name	R ₁	R ₂	pK _i
3	Clobenpropit	4-Cl-benzyl	H	9.6±0.1
22	VUF 5224	4-Cl-benzyl	methyl	8.5±0.1
23	VUF 5225	4-Cl-benzyl	ethyl	8.1±0.1
24	VUF 5228	4-Cl-benzyl	cyclo-hexyl	9.3±0.2
25	VUF 5642	cyclo-hexyl	cyclo-hexyl	7.9±0.1

Substitution of the isothiurea group of clobenpropit by a methyl or ethyl group results in a significant decrease of H₃ affinity (table 2). The electron donating properties of these small alkyl groups have the anticipated disadvantageous effect on the interaction of the isothiurea moiety with the two site points C and D (*vide supra*). However, with the cyclohexyl-substituted compound **24** the high H₃ affinity (pK_i=9.3±0.2) is regained. Clearly, for this compound the electron donating effect of the extra cyclo-alkyl substituent is compensated by the interaction with the second hydrophobic pocket. The effect on the H₃ affinity of electron releasing substituents on the isothiurea moiety is further illustrated by compound **25** (pK_i=7.9±0.1), as the disadvantageous effect of two electron-releasing cyclohexyl groups is not compensated by the interaction with the two distinct hydrophobic pockets. This series of compounds validates the model that the H₃ receptor can accommodate antagonists which have two lipophilic moieties in their imidazole side chain and illustrates the different features that affect H₃ affinity.

Table 3. Histamine H₃ receptor binding affinity of the non-imidazole compounds.

				
compound	name	R ₁	R ₂	pK _i
26	VUF 5649	4-Cl-benzyl	methyl	<4
27	VUF 5650	4-Cl-benzyl	cyclo-hexyl	5.3±0.07

The deletion of the imidazole heterocycle proves to be disastrous for H₃ affinity (table 3). These results again illustrate the major influence of the imidazole nucleus on the H₃ receptor affinity of ligands at this binding site, as was also revealed in chapter 7. Interestingly, replacement of the methyl group of **26** by a cyclohexyl group, leading to **27**, results in a better H₃ ligand. A similar improvement can be seen when comparing the imidazole-containing compounds **22** and **24**. However, it has to be noted that straightforward comparison of non-imidazole ligands **26** and **27** and the imidazole-containing compounds **22** and **24** is ambiguous as the non-imidazole compounds might interact with a different binding site of the H₃ receptor.

Conclusions

Using newly developed molecular modelling techniques, a pharmacophore model for histamine H₃ antagonists has been derived. The binding site of the histamine H₃ receptor is characterised by four hydrogen-bonding site points and two lipophilic pockets. Although qualitative by necessity, this study gives an explanation of the observed differences in SAR found for H₃ receptor antagonists. The new model has been used to design new antagonists that interact with all four hydrogen-bonding site points and the two distinct lipophilic pockets simultaneously. The compounds display the anticipated electronic and hydrophobic effects. These results illustrate that the model can be used to design novel classes of antagonists.

Experimental Section

Chemistry

General Procedure.

¹H and ¹³C NMR spectra were recorded on a Bruker AC-200 spectrometer (unless indicated otherwise) with tetramethylsilane as an internal standard. Melting points were determined on an Electrothermal IA9200 apparatus and are uncorrected. Solvents were purified and dried by standard procedures. 4-(3-Bromo-propyl)-1*H*-imidazole hydrobromide (17) was prepared according to literature procedure.^{33,34}

N-Methyl-N'-(4-chlorobenzyl)thiourea (18)

To a solution of methyl isothiocyanate (2.19 g, 30.0 mmol) in anhydrous diethyl ether (25 ml) was added dropwise a solution of 4-chlorobenzylamine (4.25 g, 30.0 mmol) in anhydrous diethyl ether (25 ml). After vigorously stirring the suspension for 2 hours, the precipitated product was collected by filtration, washed with anhydrous diethyl ether (20 ml) and dried. A white solid was isolated (5.65 g, 88%) which was pure by TLC (eluent CHCl₃) and was used without further purification. An analytical sample was recrystallised from toluene. Mp 109.5-110.5 °C. ¹H NMR (CDCl₃): δ 2.96 (d, *J*=5.3 Hz, 3H), 4.67 (d, *J*=6.0 Hz, 2H), 6.18 (bs, 2H), 7.18-7.24 (m, 4H).

N-(4-Chlorobenzyl)-N'-ethylthiourea (19)

Analogous to the preparation of 18, using ethyl isothiocyanate.

Recrystallised from toluene, yield 90%. Mp 100-101 °C. ¹H NMR (CDCl₃): δ 1.19 (t, *J*=6.7 Hz, 3H), 3.28-3.48 (m, 2H), 4.65 (d, *J*=6.0 Hz, 2H), 5.95 (bs, 1H), 7.18-7.24 (m, 4H).

N-(4-Chlorobenzyl)-N'-cyclohexylthiourea (20)

Analogous to the preparation of 18, using cyclohexyl isothiocyanate.

Recrystallised from toluene, yield 76%. Mp: 115.5-116 °C. ¹H NMR (CDCl₃): δ 0.95-2.10 (m, 10H), 3.80 (m, 1H), 4.58 (d, *J*=6.0 Hz, 2H), 5.90 (bs, 1H), 6.28 (bs, 1H), 7.10-7.30 (m, 4H).

N,N'-Biscyclohexylthiourea (21)

Analogous to the preparation of 18, using cyclohexylamine and cyclohexyl isothiocyanate.

Recrystallised from toluene, yield 24%. Mp 180-182 °C.

¹H NMR (CDCl₃): δ 1.02-2.10 (m, 20H), 3.80 (m, 2H), 5.64 (bs, 2H).

N-(4-Chlorobenzyl)-S-[3-(4(5)-imidazolyl)propyl]-N'-methyl isothioureia dihydrobromide (VUF 5224, 22)

A solution of **18** (2.70g, 10.0 mmol) and 4-(3-Bromo-propyl)-1*H*-imidazole hydrobromide (**17**) in ethanol (25 ml) was refluxed for 48 hours. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography (acetate/methanol, 3/1, v/v). The product was precipitated in acetone to give a white solid (0.85 g, 18%). Mp. 163.0-164.0 °C. ¹H NMR (DMSO-d₆): δ 1.80-2.08 (m, 2H), 2.73 (t, J=7.5 Hz, 2H), 3.05 (s, 3H), 3.22-3.50 (m, 3.8 H), 4.64 (s, 2H), 7.30-7.52 (m, 5H), 9.04 (s, 1H). Anal. calcd. for C₁₅H₂₁Br₂ClN₄S: C 37.17; H, 4.37; N, 11.56. Found: C, 37.16; H, 4.50; N, 11.29.

N-(4-Chlorobenzyl)-N'-ethyl-S-[3-(4(5)-imidazolyl)propyl]isothioureia dihydrobromide (VUF 5225, 23)

Analogous to the preparation of **22**, using **19**. The product was isolated as an oil (25%).

¹H NMR (DMSO-d₆): δ 1.20 (t, J=7.3 Hz, 3H), 1.75-2.03 (m, 2H), 2.71 (t, J=7.5 Hz, 2H), 3.10-3.65 (m, 5.8 H), 4.66 (s, 2H), 7.20-7.58 (m, 5H), 8.98 (s, 1H). Anal. calcd. for C₁₆H₂₃Br₂ClN₄S: C, 38.53; H, 4.65; N, 11.23. Found: C, 38.47; H, 4.74; N, 11.06.

N-(4-Chlorobenzyl)-N'-cyclohexyl-S-[3-(4(5)-imidazolyl)propyl]isothioureia dihydrobromide (VUF 5228, 24)

Analogous to the preparation of **22**, using **20**. After purification by column chromatography and evaporation of a uniform fraction, the semi-solid residue was suspended in dry diethylether and stirred until complete precipitation. The very hygroscopic material was filtered and dried. Yield 22%, mp: 127.0-130.0 °C.

¹H NMR (DMSO-d₆): δ 1.00-2.08 (m, 12H), 2.67 (t, J=7.5 Hz, 2H), 3.31 (t, J=7.2 Hz, 2H), 3.79 (m, 1H), 4.68 (s, 2H), 7.29-7.51 (m, 5H), 8.81 (s, 1H). Anal. calcd. for C₂₀H₂₉Br₂ClN₄S: C, 43.45; H, 5.29; N, 10.13. Found: C, 43.60; H, 5.20; N, 10.02.

N,N'-Biscyclohexyl-S-[3-(4(5)-imidazolyl)propyl]isothioureia dihydrobromide (VUF 5642, 25)

Analogous to the preparation of **22**, using **21**. The product was isolated as a white solid (18%). ¹H NMR (DMSO-d₆): δ 1.00-2.08 (m, 22H), 2.74 (t, J=7.5 Hz, 2H), 3.32 (t, J=7.0 Hz, 2H), 3.63-3.90 (m, 2H), 7.47 (s, 1H), 8.95 (s, 1H). Anal. calcd. for C₁₉H₃₄Br₂N₄S: C, 44.71; H, 6.71; N, 10.98. Found: C, 44.56; H, 5.73; N, 10.94.

N-(4-Chlorobenzyl)-N'-methyl-S-propyl]isothioureia hydrobromide(VUF 5649, 26)

Analogous to the preparation of **22**, using **18** and 1-bromopropane. The crude product was purified by chromatography (CHCl₃/methanol, 95/5, v/v). A white product was isolated

(43%). Mp 107.5-108.5 °C. ¹H NMR (CDCl₃) : δ 0.96 (t, J=7.5 Hz, 3H), 1.50-1.75 (m, 2H), 3.04 (s, 3H), 3.47 (t, J=6.0 Hz, 2H), 4.59 (s, 2H), 7.60 (br s, 1H), 7.14-7.38 (m, 4H), 9.70 (bs, 1H). Anal. calcd. for C₁₂H₁₈BrClN₂S: C, 42.68; H, 5.37; N, 8.29. Found: C, 42.64; H, 5.29; N, 8.37.

N-(4-Chlorobenzyl)-N'-cyclohexyl-S-propyl]isothiourea hydrobromide (VUF 5650, 27)

Analogous to the preparation of **26**, using **20**. A white solid was obtained (43%). Mp=146.0-147.0 °C. ¹H NMR (CDCl₃) : δ 0.94 (t, J=7.34 Hz, 3H), 1.03-1.93 (m, 10H), 1.51-1.68 (m, 2H), 3.11 (t, J=5.34 Hz, 2H), 3.64-3.88 (m, 1H), 4.67 (s, 2H), 6.28 (bs, 1H), 7.12-7.41 (m, 4H). Anal. calcd. for C₁₇H₂₆BrClN₂S: C, 50.31; H, 6.46; N, 6.90. Found: C, 50.19; H, 6.71, N, 6.98.

Pharmacology

The affinity for the histamine H₃ receptor was determined as described in chapter 2.

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Summary

Synthesis, structure-activity relationships and molecular modelling of histamine H₃ receptor ligands

The research described in this thesis concerns the molecular features that are involved in the interaction of the histamine H₃ receptor with its ligands. As molecular biological data and structural information of the H₃ receptor are scarce, the way to describe ligand-receptor interaction is to study the characteristics of the H₃ ligands. Thus, the structure-activity relationships (SARs) of H₃ agonists and antagonists form the premise of the work described in this thesis, and a thorough review of all known H₃ ligands is therefore given in chapter 1.

The scope of this thesis is defined in chapter 2 and it is concluded that iterative and synergistic cycles of synthesis, pharmacological evaluation and molecular modelling of H₃ ligands can improve the understanding of the molecular features of H₃ ligands that determine their affinity and efficacy. In chapter 3, the synthesis of the small and rigid histamine analogue 2-(1*H*-Imidazol-4-yl)cyclopropylamine is described. Various attempts to synthesise this compound via non-concerted or metal-catalysed cyclopropanation reactions of imidazole-containing precursors suffered from serious drawbacks. However, an alternative synthetic approach, constructing the imidazole ring on an appropriate cyclopropyl precursor, has enabled the synthesis and resolution of *trans*-2-(1*H*-Imidazol-4-yl)cyclopropylamine. All attempts to prepare *cis*-2-(1*H*-imidazol-4-yl)cyclopropylamine have failed. Pharmacological evaluation of the *trans*-2-(1*H*-imidazol-4-yl)cyclopropylamine enantiomers, using different assays, has revealed moderate agonistic activity on the H₃ receptor. The stereoisomer (1*S*, 2*S*)-2-(1*H*-Imidazol-4-yl)cyclopropylamine (VUF 5297) is established as the eutomer. These findings are rationalised in the subsequent chapter.

In chapter 4, a pharmacophore model is constructed that describes the interaction of both H₃ agonists and antagonists with a putative aspartic-acid residue (Asp) of the receptor. In these molecular modelling studies, it is presumed that the H₃ receptor binds the imidazole ring of the ligands in a compulsory manner, allowing the superposition of the imidazole ring of the ligands. Furthermore, it is assumed that the protein backbone that contains the interacting Asp

is rigid; hence, the C α and C β carbon atoms of the interacting residue always occupy the same position in 3D space. The geometries of the pharmacon-Asp complexes are investigated using an accurate density functional approach. It is revealed that the conformation of the Asp uniquely discriminates between binding agonists and antagonists, providing a molecular determinant that describes efficacy. In addition, the model can qualitatively explain the stereoselectivity of the receptor (also observed in chapter 3). Furthermore, two hydrophobic pockets available for binding H₃ antagonists are indicated. These results explain the differences in SAR observed for the different classes of antagonists. Due to the computational methods used, a detailed description of the position and shape of these hydrophobic pockets of the receptor could not be obtained. Another limitation of the methods used is that the binding mode of H₃ antagonists that lack a basic nitrogen atom in their imidazole side chain cannot be investigated.

In chapter 5, the synthesis and pharmacological evaluation of a new and potent H₃ receptor antagonist 4-[2-(1H-imidazol-4-yl)-ethyl]-piperidine (VUF4929) is reported. The ligand reveals remarkable discrepancies between the different pharmacological assays, thereby suggesting the presence of H₃ receptor heterogeneity. The compound may become a valuable tool in future pharmacological studies. Alkylation of the basic nitrogen atom in the piperidine ring of VUF 4929 with lipophilic moieties results in compounds with reduced H₃ activity, as predicted by the qualitative model described in chapter 4.

Chapter 6 describes efforts to synthesise new and rigid H₃ antagonists that could serve as templates in additional molecular modelling studies. Using the model that is described in chapter 4, it was predicted that specific modifications of a potent H₃ agonist should result in conformationally restrained antagonists. However, having synthesised the compounds it was shown that the ligands have partial agonistic activity. These results reveal another limitation of the model that is described in chapter 4 as the ligands could only be fitted into the antagonist pharmacophore.

In chapter 7, the synthesis of two new series of H₃ ligands is described. Pharmacological testing of these 4-(*n*-alkyl)-1H-imidazoles and the 4-(ω -phenylalkyl)-1H-imidazole series show the contribution of lipophilic moieties in the imidazole side chain on the H₃ activity. The compounds also indicate the relative location of a hydrophobic pocket with respect to the imidazole-binding pocket of the receptor. In addition, the influence of a basic nitrogen atom in the imidazole side chain in other classes of antagonists can now be evaluated. These results and the development of a new molecular modelling tool that allows the superposition of two flexible ligands have enabled the construction of a more detailed pharmacophore model for

H₃ antagonists (chapter 8). In this model, the H₃ receptor antagonist binding site is described by four hydrogen-bonding site points and two hydrophobic pockets. In the model, the binding mode of all H₃ antagonists is described (with the exception of recently described non-imidazole ligands) and explains the SAR of all classes of antagonists qualitatively. Furthermore, the design, synthesis and pharmacological testing of a small series of new and potent H₃ antagonists gives further proof of concept of the theoretical model.

Samenvatting

Synthese, structuur-activiteitsrelaties en moleculaire modellering van histamine H₃ liganden.

Histamine receptoren reguleren zeer uiteenlopende processen in het lichaam. Zo zijn deze receptoren onder andere betrokken bij het optreden van allergische reacties, het produceren van maagzuur en spelen ze een rol in een groot aantal processen in het centraal zenuwstelsel. Er zijn verschillende typen histamine receptoren die elk hun eigen rol en functie hebben. Door selectieve verbindingen te maken die reageren met slechts één type histamine receptor kunnen afzonderlijke effecten beïnvloed worden. Zo zijn stoffen die de histamine H₁ receptor blokkeren (H₁ antagonisten) verkrijgbaar als medicijn tegen allergische reacties (de zogenaamde antihistaminica). H₂ antagonisten worden veel voorgeschreven bij de behandeling van maagzweren.

Begin jaren tachtig werd nog een derde type histamine receptor, de H₃ receptor, ontdekt. Tot op heden is niet helemaal duidelijk of stoffen die met deze receptor reageren klinisch nuttige effecten veroorzaken. Voorlopige studies wekken de indruk dat verbindingen die de H₃ receptor activeren (H₃ agonisten) mogelijk astma kunnen bestijden en dat H₃ antagonisten misschien toepasbaar zijn als medicijn tegen de ziekte van Alzheimer of in te zetten zijn bij het “aandachtstekortstoornis-met-hyperactiviteit-syndroom”. Onderzoek naar de toepasbaarheid van H₃ liganden is in grote mate afhankelijk van de beschikbaarheid van potente en selectieve stoffen die met de H₃ receptor reageren. Om nieuwe effectieve H₃ liganden te kunnen ontwikkelen is het essentieel dat begrepen wordt waarom bepaalde stoffen interactie hebben met de H₃ receptor en ook waarom andere stoffen juist niet met de receptor reageren.

De studies die zijn beschreven in dit proefschrift zijn gericht op het verkrijgen van een beter inzicht in de moleculaire aspecten van de interactie tussen de histamine H₃ receptor en H₃ liganden. Omdat er nagenoeg niets bekend is over de structuur van de receptor kan de interactie tussen ligand en receptor alleen worden beschreven aan de hand van de H₃ liganden. Daarom wordt in hoofdstuk 1 een gedegen overzicht gegeven van alle H₃ agonisten en H₃ antagonisten en worden de structuur-activiteitsrelaties van deze liganden uiteengezet.

Het doel van het onderzoek wordt beschreven in hoofdstuk twee. Er wordt geconcludeerd dat een iteratief proces van synthese en modellering studies kan leiden tot meer inzicht in de

moleculaire herkenning van de H₃ liganden met de H₃ receptor. In hoofdstuk 3 wordt de synthese en farmacologische activiteit van het rigide histamine-derivaat 2-(1*H*-Imidazol-4-yl)cyclopropylamine beschreven. Omdat deze structuur twee chirale centra bevat bestaan er vier verschillende stereo-isomeren van dit molecuul. Alleen de twee *trans*-isomeren zijn geïsoleerd. Farmacologische studies hebben aangetoond dat deze verbindingen een matige activiteit hebben en dat de stereo-isomeer (1*S*, 2*S*)-2-(1*H*-Imidazol-4-yl)cyclopropylamine (VUF 5297) de betere agonist is. Deze waarnemingen worden generaliseerd in het volgende hoofdstuk.

In hoofdstuk 4 wordt een model voor de farmacofoor geconstrueerd dat de interactie beschrijft van zowel H₃ agonisten als H₃ antagonisten met een aminozuur (aspartaat, Asp) van de receptor. In deze moleculaire modellering studies wordt aangenomen dat de imidazool-ring van alle liganden precies op dezelfde plaats aan de receptor bindt. Verder wordt verondersteld dat de C α en C β atomen van de Asp altijd dezelfde relatieve positie hebben ten opzichte van de imidazoolring van de bestudeerde liganden. De geometrie van de ligand-Asp complexen is onderzocht door met behulp van een krachtige parallelle computer een nauwkeurige minimalisatie-methode toe te passen. Het model toont aan dat de conformatie van de Asp verschilt wanneer er een interactie met een agonist plaatsvindt en wanneer een antagonist gebonden wordt. De stereoselectiviteit van de receptor kan kwalitatief worden verklaard. Verder suggereert het model dat er twee verschillende lipofiele holtes in de H₃ receptor zijn waarmee lipofiele delen van H₃ antagonisten interactie kunnen hebben. Dit laatste zou het verschil in structuur-activiteitsrelaties tussen de diverse klassen van antagonisten kunnen verklaren. Helaas is het met de gebruikte methode niet mogelijk om de positie en vorm van de lipofiele holte gedetailleerd te beschrijven. Ook de interactie van antagonisten die geen basisch stikstofatoom in de imidazool-zijketen hebben kan binnen dit model niet worden onderzocht.

In hoofdstuk 5 wordt de synthese en activiteit van een nieuwe H₃ antagonist, 4-[2-(1*H*-imidazol-4-yl)-ethyl]-piperidine (VUF4929) beschreven. De verbinding toont opmerkelijke verschillen aan tussen de farmacologische systemen waarop H₃ activiteiten worden vastgesteld. Dit resultaat geeft mogelijk aan dat met behulp van deze farmacologische systemen het effect van de liganden op meer dan één (unieke) receptor gemeten wordt. Toekomstige farmacologische studies zijn noodzakelijk om dit fenomeen nader te onderzoeken; VUF 4929 zou hierbij een belangrijk hulpmiddel kunnen zijn. Verder wordt in dit hoofdstuk beschreven dat alkylering van het basisch stikstofatoom in de piperidine-ring van VUF 4929 leidt tot derivaten die minder goed binden aan de H₃ receptor. Dit resultaat

was voorspeld (en is dan ook in overeenstemming) met het model dat is beschreven in hoofdstuk 4.

In het zesde hoofdstuk worden pogingen beschreven om rigide H_3 antagonisten te ontwikkelen die nuttig zouden kunnen zijn bij de constructie van een meer gedetailleerd farmacofoor model voor H_3 antagonisten. Het model dat is beschreven in hoofdstuk 4 voorspelt dat bepaalde veranderingen aan de structuur van een H_3 agonist zullen leiden tot nieuwe H_3 antagonisten. Na synthese van de stoffen is aangetoond dat de verbindingen partiële agonisten zijn. Daarmee onthullen de liganden nog een tekortkoming van het model dat is beschreven in hoofdstuk 4, omdat deze verbindingen alleen in de farmacofoor voor antagonisten kunnen worden gefit.

In hoofdstuk 7 wordt de ontwikkeling van twee nieuwe series H_3 antagonisten behandeld. Farmacologische evaluatie van deze 4-(*n*-alkyl)-1*H*-imidazolen en 4-(ω -phenylalkyl)-1*H*-imidazolen toont aan in hoeverre de lipofiele groepen van H_3 antagonisten bijdragen aan de H_3 activiteit. Indirect wordt daarmee ook het effect van een basisch stikstofatoom in de imidazool-zijketen op de H_3 activiteit aangetoond. Verder leveren de verbindingen belangrijke informatie omtrent de relatieve positie van een lipofiele pocket van de receptor ten opzichte van de plek waar de receptor de imidazool-ring van de liganden bindt. Deze resultaten maken het, samen met de ontwikkeling van een nieuw moleculair modelling programma, mogelijk om een meer gedetailleerd farmacofoor model voor H_3 antagonisten te maken (hoofdstuk 8). In dit laatste model wordt de H_3 antagonistbindingsplaats gekarakteriseerd door vier punten die waterstofbruggen met de liganden kunnen vormen en twee lipofiele pockets die lipofiele delen van de liganden kunnen binden. De interactie van alle H_3 liganden met de receptor is beschreven (met uitzondering van de recentelijk ontdekte niet-imidazool liganden) en de structuur-activiteitsrelaties kunnen kwalitatief worden verklaard. De design, synthese en farmacologische evaluatie van een kleine serie nieuwe en potente H_3 antagonisten geven een verdere validatie en onderbouwing van dit theoretische model.

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Curriculum Vitae

Iwan de Esch was born in Heerlen, the Netherlands, on May 26, 1970. In 1987 he completed his secondary education and from 1987-1991 he studied organic chemistry at the Limburg University for Professional Education. The last year of these studies was performed at the University of Nijmegen where he did research in the field of supramolecular chemistry in the group of Prof. Dr R. J. M. Nolte. From 1991-1994 he studied organic chemistry at the University of Nijmegen and was involved in a research project concerning asymmetric cycloaddition reactions in the group of Dr H. Scheeren. After receiving his M.Sc., he started his Ph.D. research at the department of Medicinal Chemistry, Vrije Universiteit Amsterdam (Prof. Dr H. Timmerman) on a project that resulted in this thesis. Since 1998 he has been a research associate at the Drug Design Group (Dr P. Dean) of the University of Cambridge, UK. He is a co-founder of De Novo Pharmaceuticals, a start-up company to be spun out of the University of Cambridge that applies drug design to the novel therapeutic targets emerging from genomics.

